EVALUATION OF ANTIDEPRESSANT EFFECT OF VARIOUS STATINS IN COMPARISION WITH FLUOXETINE IN SWISS MALE ALBINO MICE

DISSERTATION SUBMITTED FOR THE DEGREE OF

M.D BRANCH –VI

PHARMACOLOGY

MAY – 2018

THE TAMILNADU

Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI.

TAMILNADU.

Madurai

10. 2017

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIDEPRESSANT EFFECT OF VARIOUS STATINS IN COMPARISION WITH FLUOXETINE IN SWISS MALE ALBINO MICE"** is a bonafide record of work done by **Dr.V.Vinothini,** under the guidance and supervision of **Dr.R.SAROJINI, M.D.,** Professor, in the Institute of Pharmacology, Madurai Medical College, Madurai, during the period of her postgraduate study of M.D Pharmacology from 2015-2018.

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DECLARATION

I, **Dr.V.Vinothini** solemnly declare that the dissertation titled **"EVALUATION OF ANTIDEPRESSANT EFFECT OF VARIOUS STATINS IN COMPARISION WITH FLUOXETINE IN SWISS MALE ALBINO MICE"** has been prepared by me under the able guidance and supervision of **Dr. R. Parameswari M.D,** Director and Professor, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfilment of the regulation for the award of M.D Pharmacology degree examination of the Tamilnadu Dr.MGR Medical University, Chennai to be held in May 2018.

This work has not formed the basis for the award of any degree or diploma to me, previously from any other university to anyone.

Place: Madurai **Dr. V.VINOTHINI**

Date:

ACKNOWLEDGEMENT

I am greatly indebted to **Dr. D. MARUTHUPANDIAN, M.S., F.I.C.S., F.A.I.S.,** Dean (FAC), Madurai Medical College and Government Rajaji hospital, Madurai who initiated this interdisciplinary work with generous permission.

It is with great pleasure I record my deep respects, gratitude and indebtedness to **Dr.R.Parameswari, M.D.,** Director and Professor, Institute of Pharmacology, Madurai medical college, Madurai for her remarkable guidance, encouragement and selfless support which enabled me to pursue the work with perseverance**.** Her contagious enthusiasm was a source of energy to me in successfully completing my dissertation under her generous guidance.

I am extremely thankful to my guide **Dr.R.Sarojini**, **M.D.,** Professor, Institute of Pharmacology, Madurai Medical College, Madurai, for her valuable suggestions and critical review at every stage for the successful completion of this study.

I record my sincere and heartfelt thanks to **Dr.S.Vijayalakshmi, M.D.,** Professor of Pharmacology, Madurai Medical College, Madurai for her untiring support, continuous suggestions and enduring encouragement throughout the study.

I am thankful to **Dr.M.Shanthi, M.D.,** Professor of Pharmacology, for her valuable suggestions, enduring encouragement and constant support throughout the study.

I am thankful to **Dr.K.Raadhika, M.D.,** Associate Professor of Pharmacology, for her valuable suggestions and support. I thank **Dr.M.Padmini, B.V.Sc.,** Veterinary assistant surgeon, Central animal house, Madurai Medical College, Madurai.

It is with deep sense of gratitude, I wish to express my sincere thanks to Assistant Professors **Dr.M.SheikDavooth, M.D., Dr.V.Thaivanai, M.D., Dr.M.S.Ahil, M.D., Dr.K.Geetha, M.D., Dr.M.Malathi, M.D., Dr.B. Jayapriya, M.D., Dr.S. Sankareswari, M.D., Dr.T.Gowrithilagam, M.D.,**

I am extremely thankful to **Dr.M.Saleem, M.D.,** Associate Professor & **Dr.S.Priya, M.D.,** Assistant Professor, Institute of Preventive and Social Medicine, Madurai Medical College, Madurai for their valuable suggestions and support towards statistical work out.

It is my duty to express my appreciation to my colleagues **Dr.R.Vijayarani, Dr.G.Muthukavitha, Dr.S.Vasanth, Dr.R.Mangaladevi, Dr.S.Kiruthika, Dr.C.Uma Maheshwari, Dr.S. Prasanna Kumari, Dr.S.Meenambal Dr.XA.Prasanna, Dr.M.Priyalakshmi, Dr.S.Saraswathi, Dr. R.S. Theivanayagi, Dr.V. Sivaranjani, Dr.K. Porchelvi** for their assistance.

I thank my family members and staff members of the Institute of Pharmacology for their kind support and encouragement throughout the study.

I thank the almighty who had bestowed his mercy and kindness all throughout my study and my carrier.

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INTRODUCTION

INTRODUCTION

Depression is a chronic debilitating disease. It's a major psychotic illness characterised by intense despair, sadness, loss of concentration, mental slowing, pessimistic worry and lack of pleasure. It is associated with physical symptoms like insomnia or hypersomnia, altered eating pattern with anorexia, weight loss and over eating.[1] As in Shakessphere's words "Hell is empty and all the devils are here" it affects the quality of life in every aspect – personally, socially, financially. It affects not only the patient's life, but also the care taker's life.

It prevails in the world since time immemorial. First recorded history is about Saul, an Israeli king. In the Book of Samuel, written three thousand years ago, it was said that he was influenced by an "evil spirit" that makes him " hopeless, incapacitated, guilt-ridden" leading to his suicide.[2] Then in Greek period Hippocrates described it as melancholia: melas - black and kohle - bile based on the Greek ideology of body fluid dependent pathology.

According to World Health Organisation (WHO) over 4.4 % of global population suffer from depression.[3] it is distributed over all age groups. The burden is fifty percent more in females and it will be the second important cause of global disease burden by the year 2020. The worst part is the relevance of the data may be tip of the ice berg.

Depression is an age old disease. Although the ideology behind the disease evolved with the ages, it's still incomplete. The treatment of depression also progressed with time. In the initial period it was grim as the patients were treated in asylum. In current scenario with better comprehension of the pathogenesis there are various modalities of treatment available for the disease. The treatment modalities are pharmacological and non-pharmacological. Pharmacological intervention is the effective mode of treatment which can be supplemented by non-pharmacological interventions like cognitive behaviour therapy (CBT), computerized physical activity programme and guided self help. As the most accepted aetiology of major clinical depression is imbalance in amine neurotransmission, significant part of pharmacotherapy is based on drugs which ensure the balance in the neurotransmission of the central nervous system. So these antidepressants act by increasing the amines serotonin and noradrenalin at the neuronal synapse. The first generation antidepressants are Tricyclic Antidepressants (TCA) and Monoamine Oxidase Inhibitors (MAOIs). These drugs are not commonly preferred as they are associated with serious adverse reactions. The second generation drugs like Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin Nor adrenaline Reuptake Inhibitors (SNRIs) are the commonly preferred ones, as they are more selective, and their safety margin is definitely a notch higher than the first generation drugs [4].

Though the SSRIs & SNRIs are the commonly prescribed drugs and of proven efficacy among the available drugs, there are trials in recent time that questions their clinical effectiveness. Another major problem with these drugs is

lack of patient compliance. It is very low; almost fifty percent of the patients don't adhere to therapy[5]. It could be due to social stigma and also due to factors like cost, fear of discontinuation, drug dependence, lack of motivation and follow up. As the etiopathogenesis of major depressive illness is still evolving, there are many mysteries yet to be revealed. Any new drug which offers effectiveness is not only making itself an option for treatment but also unfold some of those mysteries. It provides new targets that also help us to understand the pathogenesis further.

On the other hand due to multifactorial reasons, hyperlipidemia and associated co-morbid conditions like cardiovascular disease, stroke, peripheral vascular disease, metabolic syndromes etc. are on the rise. Incidence of depression is found to be more associated with chronic medical conditions like coronary heart syndrome, stroke and arthritis. To prevent and to treat all such conditions, widely prescribed drug is statin. Statins have number of pleiotropic effects which may be of great value in these conditions.

Literature review on the role of statins on depression has two different views. One view is that the use of statins in coronary and other disorders are associated with some cognitive dysfunction like irritability, memory loss. This is based on the speculation that decreased cholesterol synthesis by statins interfere with receptor production. Thereby it decreases the amine mediated action in brain[6] but meta-analysis proved otherwise. Other view is statins in animal experiments are found to potentiate the antidepressant effect of fluoxetine.

In pharmacological history we have several examples of drug discovered for one purpose, during the course of time found to be a drug for treating another disease. The amine theory of depression by itself was derived accidentally when iproniazid used as anti tubercular agent was found to elevate patient's mood[7]. Statins are well known for their pleiotropic effects like improved endothelial function, neovascularisation of ischemic tissue, increasing progenitor cells, reduction of inflammation etc[8]., which gives it an inherent ability to extend its usage. These effects whether they are due to cholesterol lowering effect or due to some other effects are unknown.

As both the pathogenesis of depression and the role of statins on depression have divergent views till date, it provides lot of space for experimentation.

In this study the antidepressant effect of various statins were evaluated on animal model using male albino mice by tail suspension method.

AIM
&
OBJECTIVE

AIM

To evaluate the antidepressant effect of various statins in Swiss male albino mice by tail suspension method.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

"Raze out the written troubles of the brain
And with some sweet oblivious antidote
Cleanse the stuffed bosom of that perilous stuff
Which weighs upon the heart?"

-Shakespeare, Macbeth

As Shakespeare has said if such an antidote is present it would be easy to treat depression. But a sweet antidote is still on the search. Depression is a major mood disorder (MMD). Mood disorder includes a wide spectrum of illness, based on which side the mood swings. Manic and unipolar depressive illnesses are poles apart in the spectrum. Bipolar disorder is one in which the patient experience either elation or depression at various times. If the shift between up and down is fast, it is rapid cycling. Lower degree of mania is hypomania.

Depression is a common at the same time serious psychiatric illness; it produces profound physical and social dysfunction and also incapacitates the patient functionally. It is a disease associated with enormous health problems. Depression is a well established risk factor for medical conditions like coronary artery disease, stroke and cancer[9]. The financial ramifications of the disease are tremendous. Adding burden to the bundle is the social stigma attached to it. The disease is often misdiagnosed and undertreated. The continuum of the disease can range from nonpathological grief to major depressive disorder. So it has to be evaluated and treated effectively.

Epidemiology

Depression is one of major cause of morbidity and mortality. Worldwide over 350 million humans of all age group are sickened by depression. It forms a major component in the global load of diseases. In a survey done in 17 countries, it was detected that 1 in 20 people suffered a depressive episode within the previous year[10]. The depressive disorder peaks at second to fourth decade of a patient's life. So it reduces the functioning capacity of people grossly and makes it a leading cause of disability adjusted life year (DALY) next to cardiovascular disease. Taking the current trend of disease into consideration it will clearly overcome cardiovascular illness to top the chart. It is more common in women.

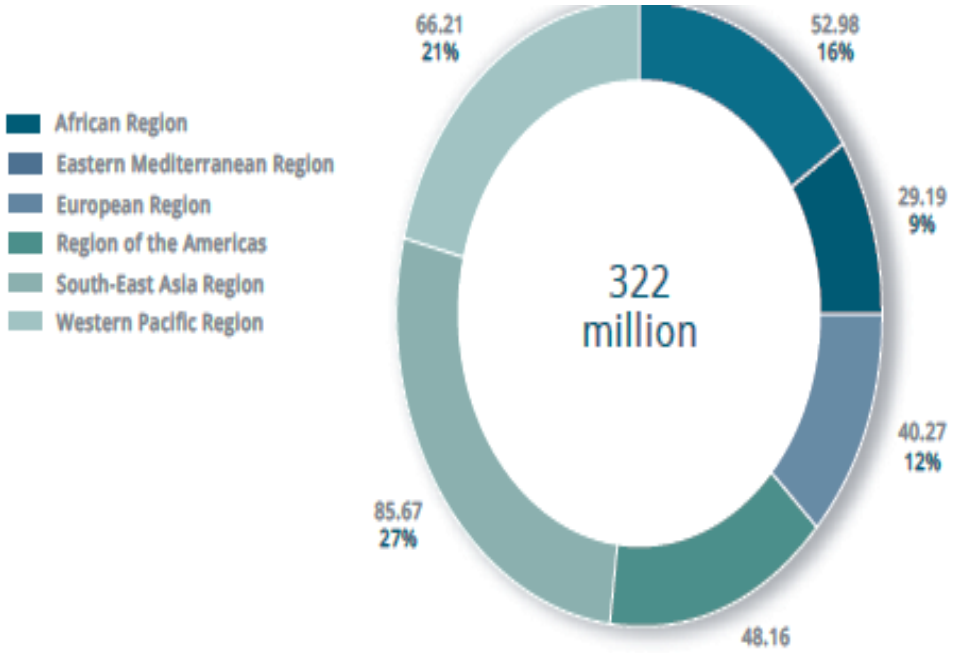


FIG1: Prevalence of depression

Post Partum Depression (PPD) is a risk factor and has a substantial influence on the poor growth of the child. The incidence of PPD in our country is around 15-20%.[11.] In Indian studies, prevalence of depression is found to be 8 to 10 per thousand population and the prevalence rates are high in the urban sector. It is on the rise among teenagers.

There is a correlation that the offspring is at the risk for depression when the parents suffer from it. The risk of depression is 2.7 fold when one parent is suffering from depression and 3 times more if both of them are suffering. Suicidal ideation is high in depressed population. 75% of the suicides are due to depression and substance abuse[12]. Suicidal incidence is more in male sex, singles, and patient with prominent symptom of hopelessness.

Etiology of depression

Depressive disorder etiology is very complex and it is difficult to totally explain it by a single social, biological or developmental factor. Several factors interact together to end up or augment the depressive disorder. Altered monoamine neurotransmission is the well proved basic pathology.

Theories of Depression

- **Biological theories**
  - Monoamine hypothesis
  - Neuroendocrine hypothesis
  - Neurotrophic hypothesis

- **Psychosocial theories**
  - Cognitive behavior theory
  - Psychodynamic theory of depression

Biological Theories

The monoamine hypothesis describes that depression is due to the functional shortfall of the monoamines 5-hydroxytryptamine (5-HT) and Nor adrenaline (NA) at certain region of the brain[13]. While mania is due to functional surplus.

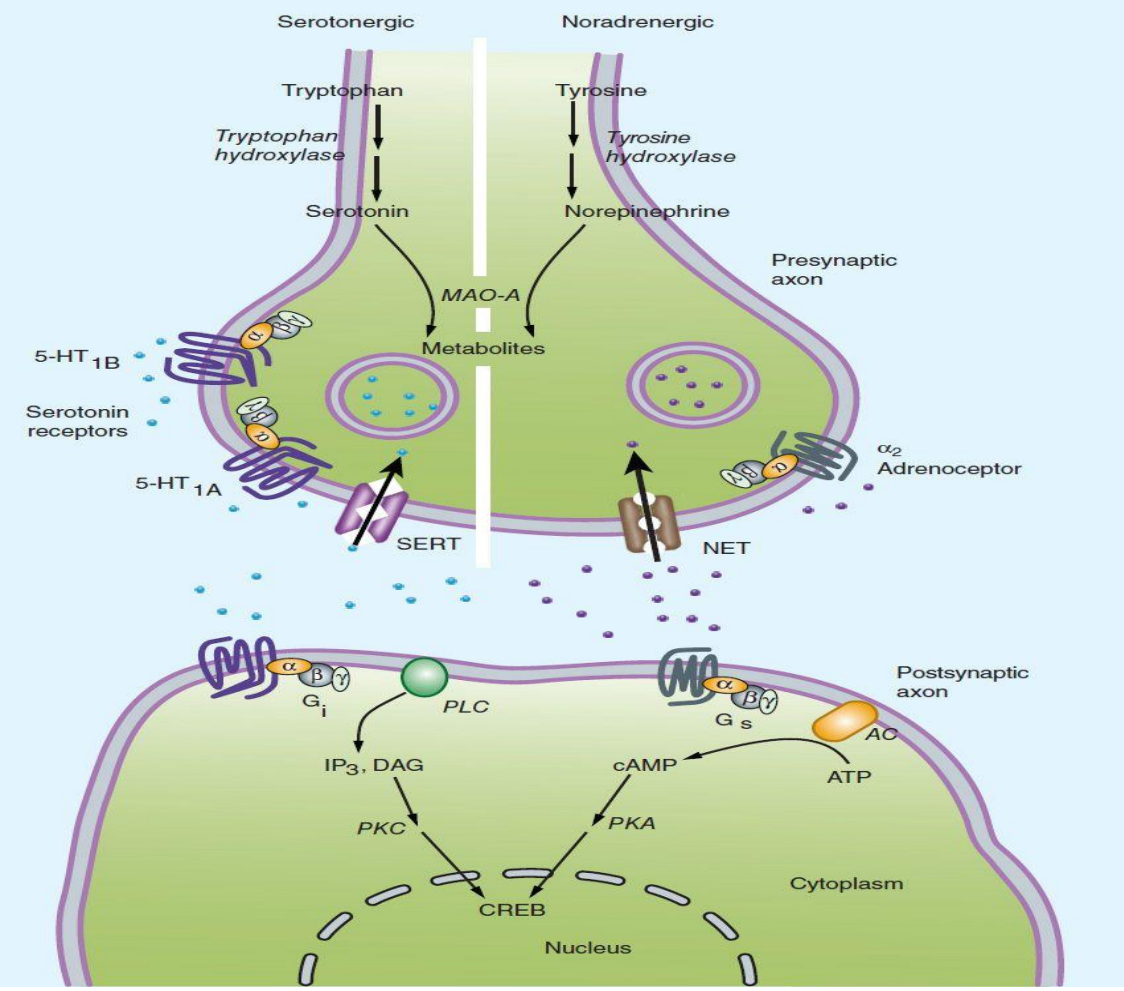


Fig 2: Monoamine transmission

The idea was accidentally discovered when the clinical effect produced by different drugs which alters the monoamine transmission in the brain improve or deteriorate the symptoms of depression. Apart from 5HT and NA, monoamines like dopamine and glutamate are also involved with depression.

This theory is strengthened by the following facts

- Pharmacological manipulation of monoamine transmission is the most successful therapeutic model
- Tricyclic antidepressants and monoamine oxidase inhibitors aid monoamine transmission.
- Drugs like reserpine cause depression.
- Serotonic activity is low in CSF of the depressed patients.
- Lack of tryptophan, a precursor of serotonin nullifies the therapeutic effect of fluoxetine an antidepressant drug.
- Anhedonia –a prime feature of depression is due to lack of dopamine neurotransmitter[14].
- Parkinson a disorder of dopaminergic pathway is frequently associated with depression.

The drawback of this theory is studies about monoamine metabolism in depressed community and studies about monoamine receptors in the postmortem brain cells of depressed cohorts gave equivocal result. Another problem is, the changes in neurotransmission that happens with depression are reverted back

within minutes to hours by antidepressants, but the therapeutic effectiveness takes longer duration of three to four weeks time.

Neuroendocrine Hypothesis [15]

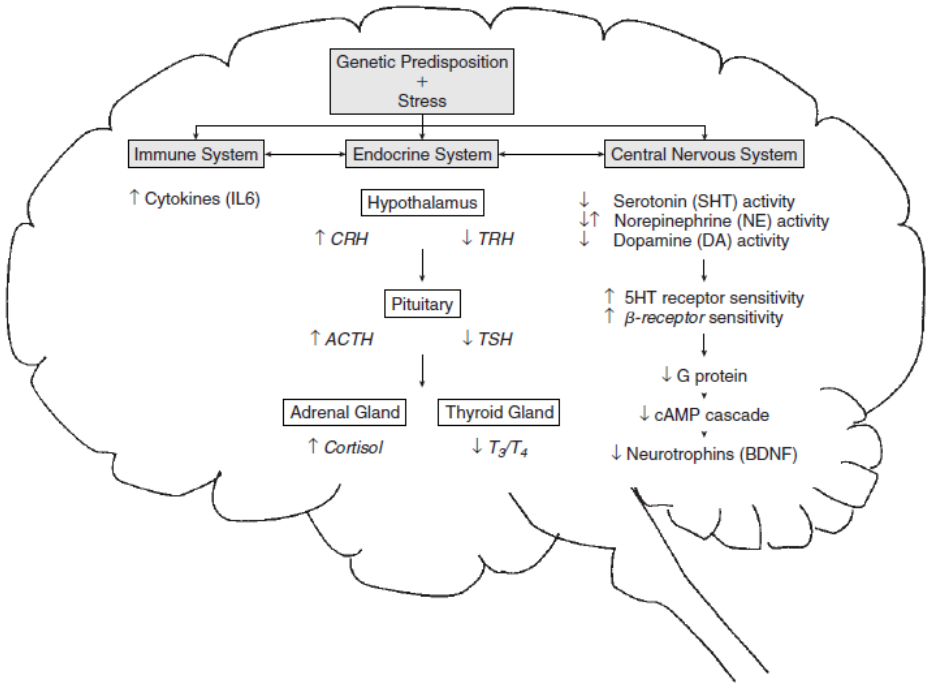


Fig 3: Endocrine abnormalities in depression

Noradrenergic and 5-HT neurotransmitters provide input to the hypothalamus. Abnormalities in these signals deregulate the release of corticotrophin-releasing hormone (CRH) and TSH releasing hormone (TRH). CRH level is increased in depressed population. CRH act on the pituitary and increase the secretion of adrenocorticotrophic hormone (ACTH). This in turn increases the cortisol level. CRH also has behavioural effects which are different from its endocrine functions. When CRH is injected in to animal their behaviour become depressed.

Neurotrophic Hypothesis[16]

Brain-Derived Neurotrophic Factor **(BDNF)** is essential for the development of neural plasticity and neurogenesis. BDNF is decreased in depression.

- Antidepressants increase the level of BDNF
- BDNF increase the neurogenesis in cortical region like hippocampus.
- Animal models exposed to chronic stress exhibit a drop in BDNF level
- Changes in the cortical region that happens during chronic stress is similar to BDNF deficiency
- Electro convulsive therapy increases the BDNF level.

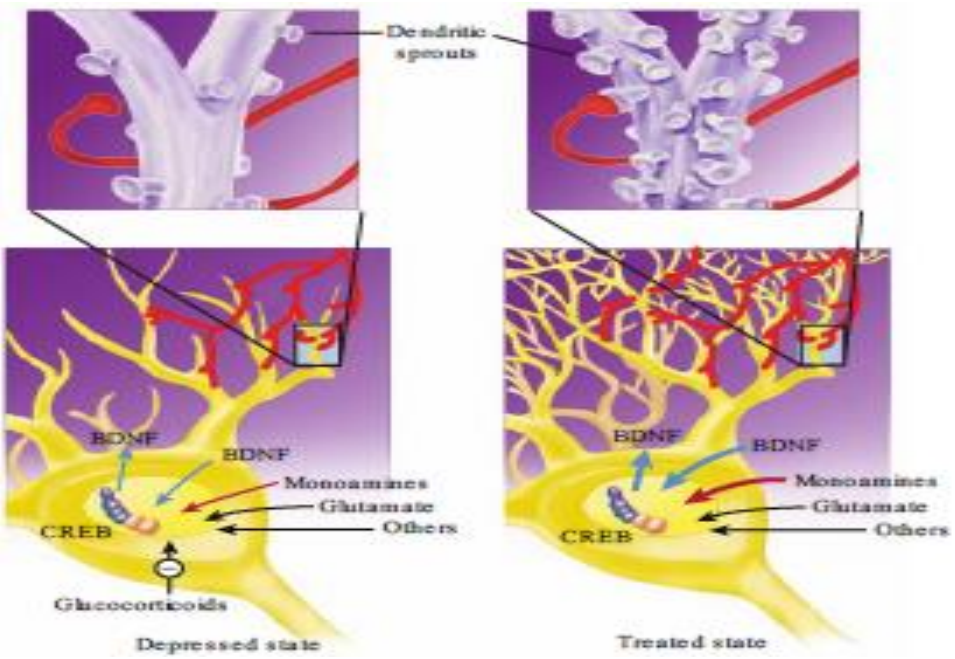


Fig 4: Role of BDNF in synaptic growth

Cortical regions where the BDNF is tropic are associated with memory and emotion like the hippocampus, cingulated gyrus and the prefrontal region.

Positron Emission Tomography scan findings associated with depression are

i) reduced activation of nucleus accumbens

ii) diminished activity in neocortical and prefrontal cortex.

iii) reduction in hippocampal size.

All these correlate with BDNF deficiency.

On the contrary the relationship between BDNF and depression is not consistent. BDNF Knockout animal models are not always associated with depressive behaviour. BDNF injected directly in to the animal's ventricle increases depressive symptoms in certain animal models. The suggested explanation for these varied findings could be due to polymorphisms among BDNF.

Psychosocial Theories

Cognitive Behaviour Theory[17]

Depression arises from cognitive maladjustment. Cognitive deregulation gives rise to misinterpreted thoughts and judgments. The severity of depression is directly proportional to degree of negative thought. Cognitive behaviour therapy is based on the helplessness-hopelessness ideology of cognitive theory.

Psychodynamic theory of depression

According to Sigmund Freud, a person must successfully resolve early developmental conflicts in order to overcome depression and achieve mental health. Mental illness is a failure to resolve this conflict.

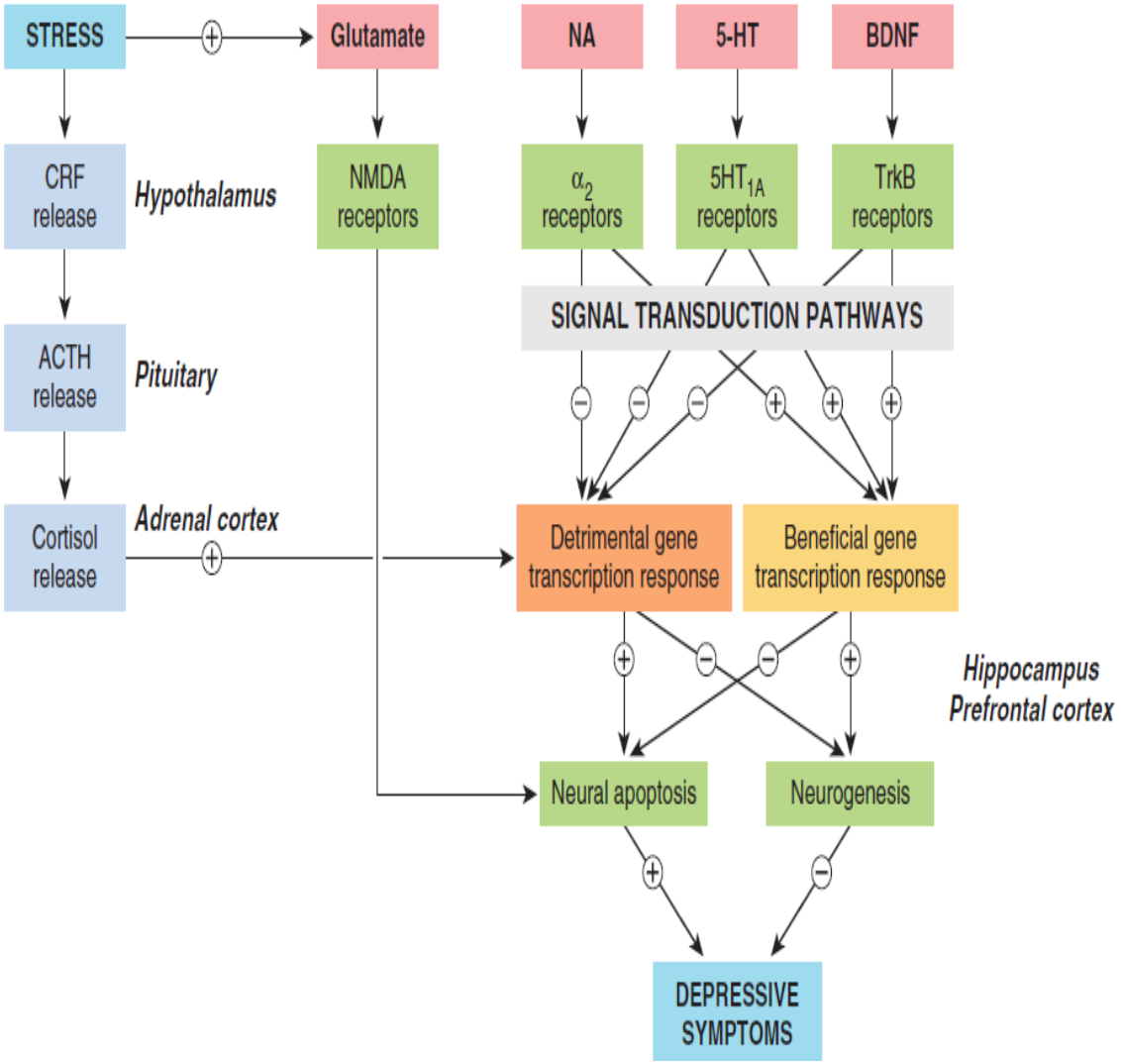


Fig 5: Pathogenesis of depression

Apart from the biological and psychosocial theories the risk factors associated with depression are [18]

- Age- Peak age of onset is second to fourth decade.
- Early parental death - Conceivable association
- Genetics - More common in monozygotic twins than dizygotic twins; common genetic disorder involving the serotonin system.
- Family history –When positive family history present there is 1.5 to 3 times increased risk.
- Marital status -Separated or divorced persons incidence is higher

  -In married males the incidence is lower

  -In married females the incidence is higher

- Negative life events – Increased association
- Personality –Anxious, compulsive, impulsive personalities are more prone.
- Physical illness- Both in neurological and non neurological illness the incidence is increased.
- Postpartum- An increased risk in the first 6-months
- Sex- Major Depression is two times common in women

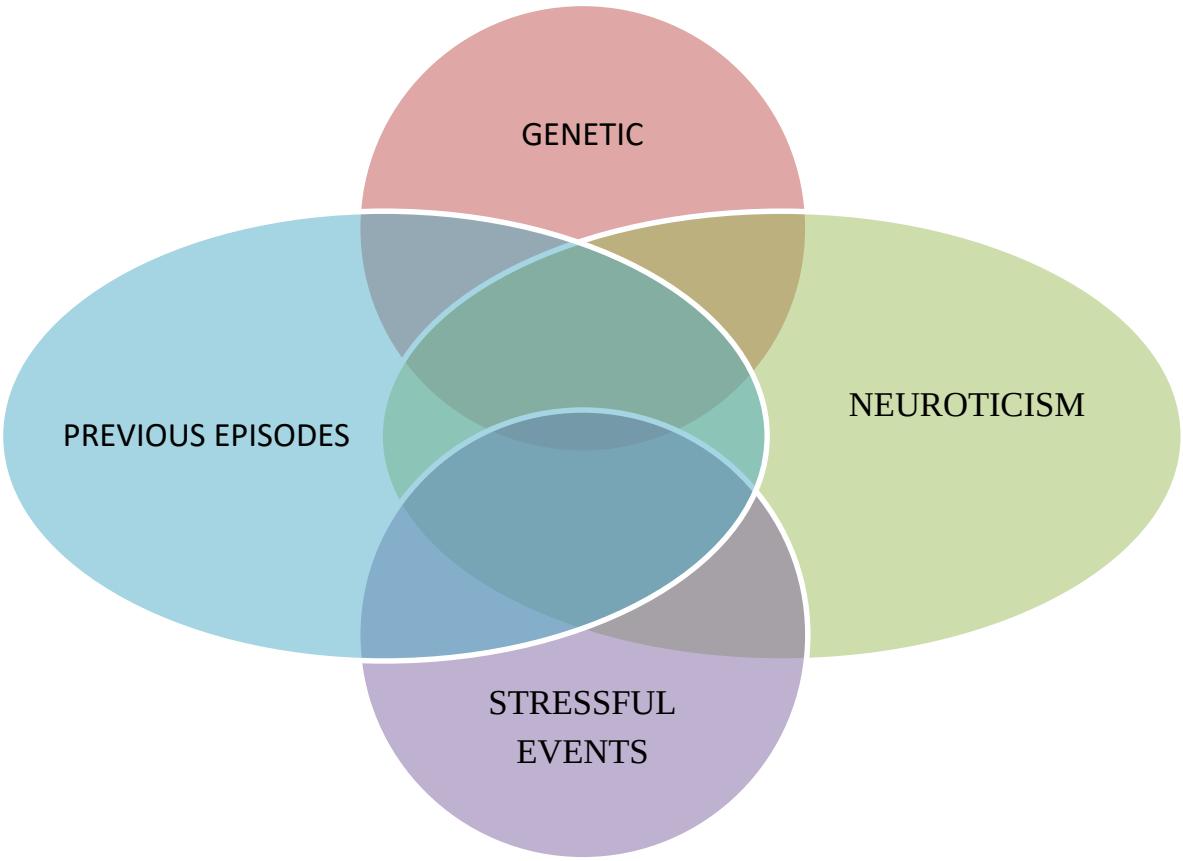


Fig 6: Integrated etiological model

- **Secondary causes of depression**
  - Drugs
  - Substance abuse
  - Medical conditions
- **Drugs**
  - Adrenocorticotropic hormone
  - Clonidine
  - Diuretics
  - Guanethidine
  - Interferon beta

- Isotreinoin
- Hydralazine
- Methyl dopa
- Oral contraceptives
- Reserpine
- Propronolol

Substance abuse

- Alcoholism
- Marijuana abuse
- Nicotine dependence
- Opiate abuse and dependence
- Psychostimulants abuse and dependence (e.g., cocaine)

Medical conditions[19]

- Endocrine abnormalities
  - Hypothyroidism
  - Addison's disease
- Deficiency states
  - Severe anemia
  - Wernicke encephalopathy
- Infections
  - AIDS

- Tuberculosis
- Sexually transmitted disease

- Metabolic disorders
  - Hypokalemia
  - Hyponatremia
  - Hepatic encephalopathy
- Cardiovascular disease
  - Hypertension
  - Congestive heart failure
  - Myocardial infarction
- Neurologic disorders
  - Alzheimer's disease
  - Epilepsy
  - Huntington disease
  - Multiple sclerosis
  - Pain
  - Post stroke
  - Parkinson's disease
- Malignancy

Mood Disorder Classification:

Based on Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) mood disorders are classified as follows

A. Depressive Disorders

1. Major Depressive Disorder, Single Episode

2. Recurrent Major Depressive Disorder,

3. Dysthymia

4. Depressive Disorder Not Otherwise Specified

B. Bipolar Disorders

1. Bipolar Disorder, Single Episode

2. Recurrent Bipolar Disorder

3. Cyclothymia

4. Bipolar Disorder Not Otherwise Specified

C. Premenstrual Dysphoric Disorder (PMDD)

(Included in DSM V.)

D. Secondary Mood Disorder Due to Non psychiatric Medical Condition

E. Substance-Induced Mood Disorder

Clinical symptoms

- **Mood**

Depression is characterized by sad mood, loss of pleasure or interest in day to day activities, social withdrawal, and decreased occupational performance.

- **Depressive ideation**

Hopelessness, helplessness, worthlessness giving rise to slowed thinking, pessimistic ideas and suicidal ideation. Severe cases are characterized by delusions of nihilism.

- **Psychomotor activity**

Decreased energy, monotonous voice, agitation, restlessness, anxiety, irritability and frustration

- **Physical symptoms**

Fatigue, chronic body aches,

- **Biological functions**

Excess or decreased sleep, loss of or excess appetite with weight modifications accordingly, and loss of libido. These changes in biological function is called somatic syndrome.

- **Suicidal risk**

Suicidal risk is found to be the most consistent symptom of depression, especially in male loners. It will be present throughout the depressive episode. Borderline personality disorders increase the risk of suicide. Mostly completed suicide is not preceded by suicidal attempts. So every patient irrespective of their previous episode is at risk of endangering their life.

Other syndromes of depression [20]

- **Involutional melancholia**

  Occur in old age associated with excess somatic symptoms, hallucination and delusion.

- **Mixed anxiety- depressive disorder**

  More common, neither fit into depression, nor into anxiety

- **Masked depression**

  Depression is masked by somatic symptoms like chronic pain, paresthesia, and alcohol abuse

- **Depressive equivalents**

  Agoraphobia, panic attacks, hysteria, obsessive compulsive disorder.

- **Double depression**

  Major depressive episode superimposed on dysthymia

- **Seasonal mood disorder**

  Bipolar or unipolar depression tends to occur in the same season.

Diagnosis of Depression[21]

For the diagnosis of major depressive episode five of the above mentioned symptoms must be present; out of this one must be sad mood and loss of pleasure and the symptoms must persist for two weeks.

The symptoms due to stressful event should be ruled out.

There should not be a manic or hypomanic episode.

After diagnosing major depressive illness it has to be categorized based on the

i) Severity of symptoms

- Mild
- Moderate
- Severe

ii) Recurrent or single episode:

When the episodes are separated by a period of two months it is recurrent episode. Otherwise considered as single episode

iii) Partial or in full remission

iv) Specifiers (associated with)

with anxiety

with catatonia

with atypical features

with peripartum onset

with seasonal pattern

mood -congruent psychotic features

mood -in congruent psychotic features

So according to DSMV the diagnosis of depression should be mentioned with

- Grade of severity
- Order of episode
- Currently in remission or not
- Associated Specifiers.

Apart from the baseline haematological, renal and hepatic parameters, other adjuvant investigations are

Cortisol level assay

Thyroid level

Sleep pattern

Monoamine metabolite turnover

Scales for Depression

A depression rating scale is a psychiatric measuring instrument having descriptive words and phrases that indicate the severity of symptoms for a time period. It is used to find the severity of depression, response to treatment and assess remission. Numerous scales are there but the important ones are

1) Hamilton Depression Rating Scale

It is the commonly used scale. In 1960, Max Hamilton designed a rating scale. It contains 17 questions and according to the severity each question is given a score from 0 to 4. [22]

2) Montgomery-Asberg Depression Rating Scale

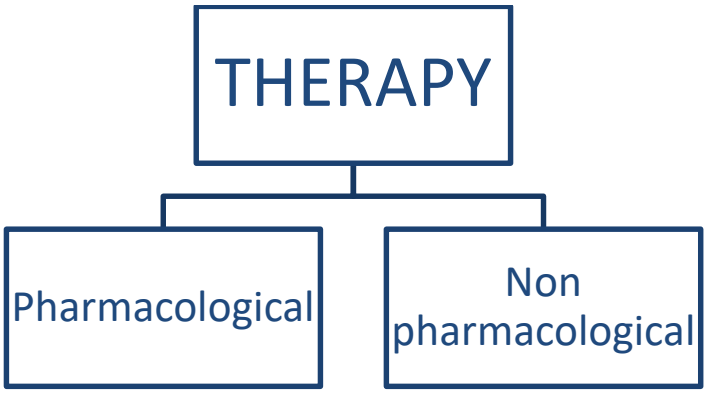
It is a questionnaire containing 10 items to be completed by researchers to assess the effects of drug therapy. It is a better scale for differentiating responders and non responders than the Hamilton Depression Rating Scale [23].

3) Raskin Depression Rating Scale

It rates severity of the symptoms in three areas: verbal reports, behaviour, and secondary symptoms of depression.

THERAPY

Pharmacotherapy forms the main mode of management. Non pharmacological management like cognitive behaviour therapy acts synergistically with pharmacotherapy. Electro Convulsive Therapy (ECT) is used in severe depression with suicidal ideations for immediate effects.



Pharmacotherapy is the common and most productive intervention in the treatment of MDD, which helps to attain remission and also prevents reoccurrence. Among the available drugs, SSRIs and SNRIs are commonly used. They belong to second generation antidepressant drugs. TCAs and MAOIs of first generation are reserved for resistant cases, and not preferred as first line because of their adverse effects.

CLASSIFICATION OF ANTIDEPRESSANTS[24]

I. Tricyclic antidepressants (TCAs)

i) Nonselective reuptake inhibition of both NA & 5HT

- Amitryptiline
- Clomipramine
- Doxepin
- Dothiepin
- Imipramine
- Trimipramine

ii) Relatively specific NA reuptake inhibition

- Amoxapine
- Desipramine
- Lofepramine
- Norclomipramine
- Nortriptiline
- nordoxepin
- Maprotiline
- Reboxetine

II. Selective Serotonin Reuptake Inhibitors (SSRIs)

- Citalopram
- Escitalopram

- Fluoxetine
- Fluvoxamine
- Paroxetine
- Sertraline

III. Serotonin and Noradrenalin reuptake inhibitors

- Desvenlafaxine
- Duloxetine
- Levomilnacipram
- Milnacipram
- Venlafaxine

IV Noradrenaline Reuptake Inhibitors

- Reboxetine

V Monoamine Receptor Antagonists

- Mirtazapine
- Trazodone,
- Mianserin

VI Monoamine Oxidase Inhibitors

i) Irreversible, non-competitive inhibitors

- Isocarboxazid
- Phenelzine,
- Tranylcypromine

ii) Reversible MAO-A-selective inhibitors (RIMA)

- Belfoxatone
- Brofarmine
- Clorgyline
- Moclobemide
- Pirilindole
- Toloxatone

VII Others

- Bupropion

Non Pharmacological Treatments

1. Psychotherapies
2. Brain stimulation techniques

1. PSYCHOTHERAPIES

- Cognitive Behavior Therapy

Cognitive behavior is the first line therapy in managing mild to moderate depression. In severe depression, CBT should be combined with pharmacotherapy for effectiveness. Its main objective is to correct the negative ideations.

- Inter Personal Therapy (IPT)

Inter personal therapy helps to improve the social functioning of the patient by exploring stressors like interpersonal disputes, transitions, skill deficit, and isolation.

Educate the family for the purpose of treatment compliance. Family focused therapy with sessions of psychoeducation, communication training and problem solving skill training for the family members to achieve better treatment compliance.

2. BRAIN STIMULATION TECHNIQUES [25]

- Electro- Convulsive Therapy (ECT)

Electro- convulsive therapy is one of the earliest treatments and is still the most effective in treating severe depression, but for a short span only. It should always be given along with antidepressants. It is preferred in patients with suicidal tendency. Bilateral ECT is more effective than unilateral ECT.

- Deep brain stimulation

Chronic white matter stimulation along the cingulated region in resistant cases

- Vagus Nerve Stimulation

An electrical device is implanted in the chest wall in the subcutaneous plane which stimulates the cortex through the left cervical vagus. Federal Drug Agency (FDA) approved this technique for resistant depression and epilepsy.

- Transcranial Magnetic Stimulation (TMS)

  It's a non invasive method of applying electrical stimulus through the scalp. Left frontal TMS gives some benefit.

- Light therapy

  Light therapy is a treatment for seasonal affective disorder. It relieves irritability associated with depression. Less effective in non seasonal depression

Non responders

As depression is a heterogeneous disorder, there are various reasons for non responding. The common non responders are old age, patient with limited economic and interpersonal resources, low quality of life, chronic depression, poor social and family setup, noncompliance and vascular depression.

- Diagnosis must be reassessed
- Hypothyroidism if present should be corrected
- Whether the patient had taken drugs for a sufficient period, so that the antidepressants would have their full effect should be assessed .

The treatment options for non responders are[26]

- optimization of antidepressants dose
- switching to other antidepressants
- combination of antidepressants
- augmentation strategies
- somatic therapies
- integrated approach
- adjunctive approach

DRUGS USED IN DEPRESSION

Selective 5-hydroxytryptamine reuptake inhibitors

SSRIs are the commonly used anti depressant drugs. They are very much effective in treating major depressive illness. TCAs remained the mainstay of drug therapy of depression for many years. To overcome the adverse affects of TCAs due to blockade of receptors other than serotonin and adrenaline there was a continuous search for a better compound. With methodological screening SSRIs were introduced based on the structure of TCAs. Though SSRIs have a single therapeutic profile of blocking the Serotonin reuptake transporter (SERT), they are chemically diverse compounds.[27]

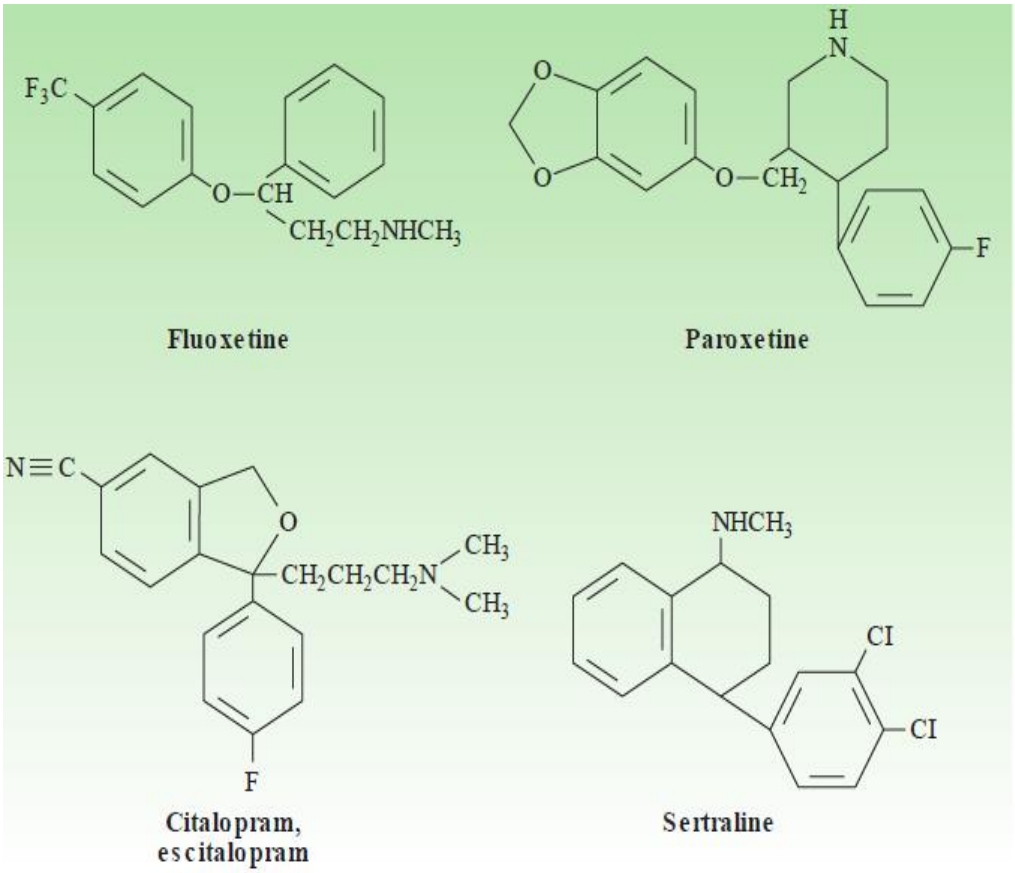


Fig 7: Structure of various SSRI

Fluoxetine

The first introduced SSRI was zimelidine, but it was associated with Guillain–Barre´ syndrome. Second one was fluvoxamine, then came fluoxetine. It is the commonly prescribed SSRI. In this study it was used as the standard drug. The primary target of SSRI is SERT. It differs from other SSRIs in its secondary pharmacological characteristics.

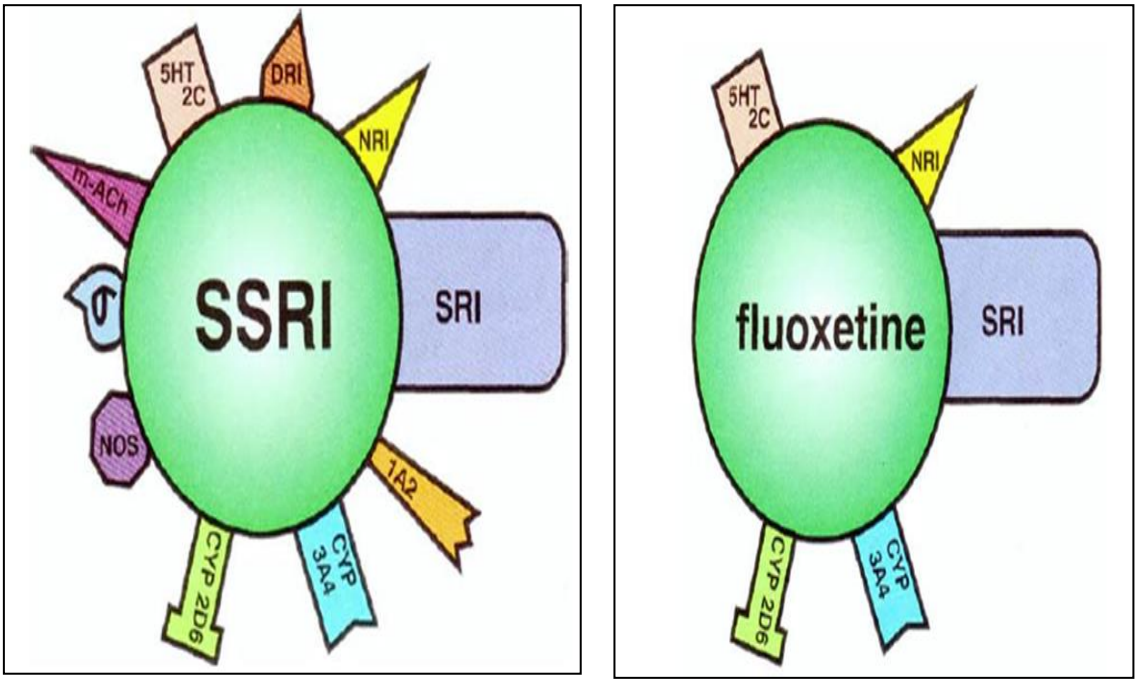
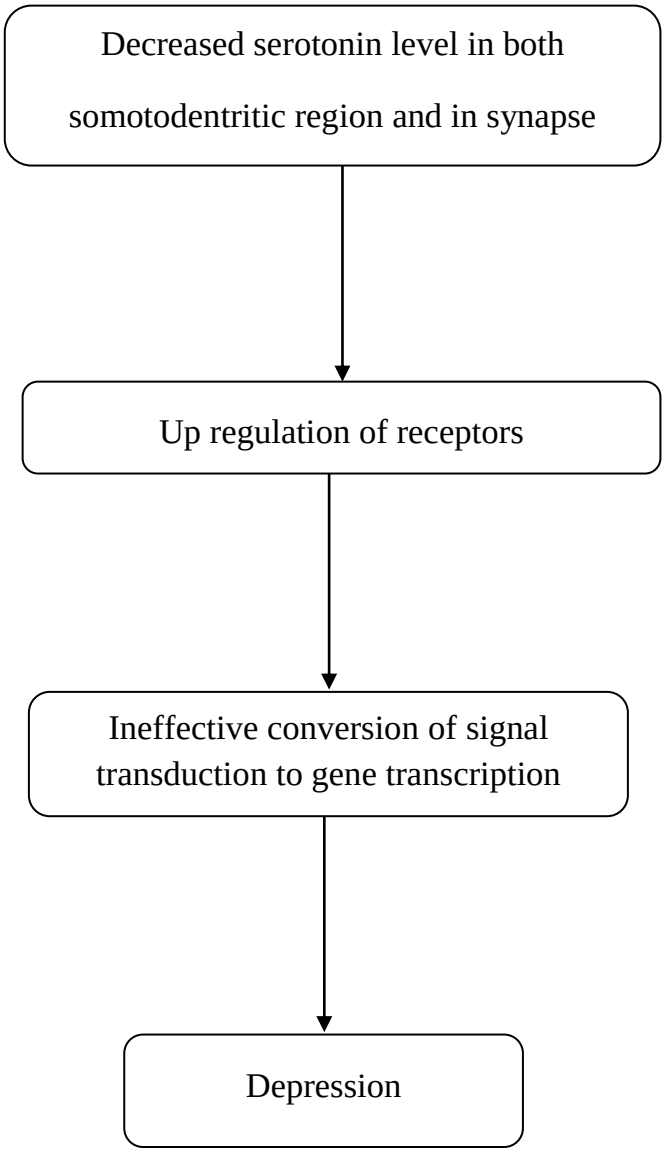


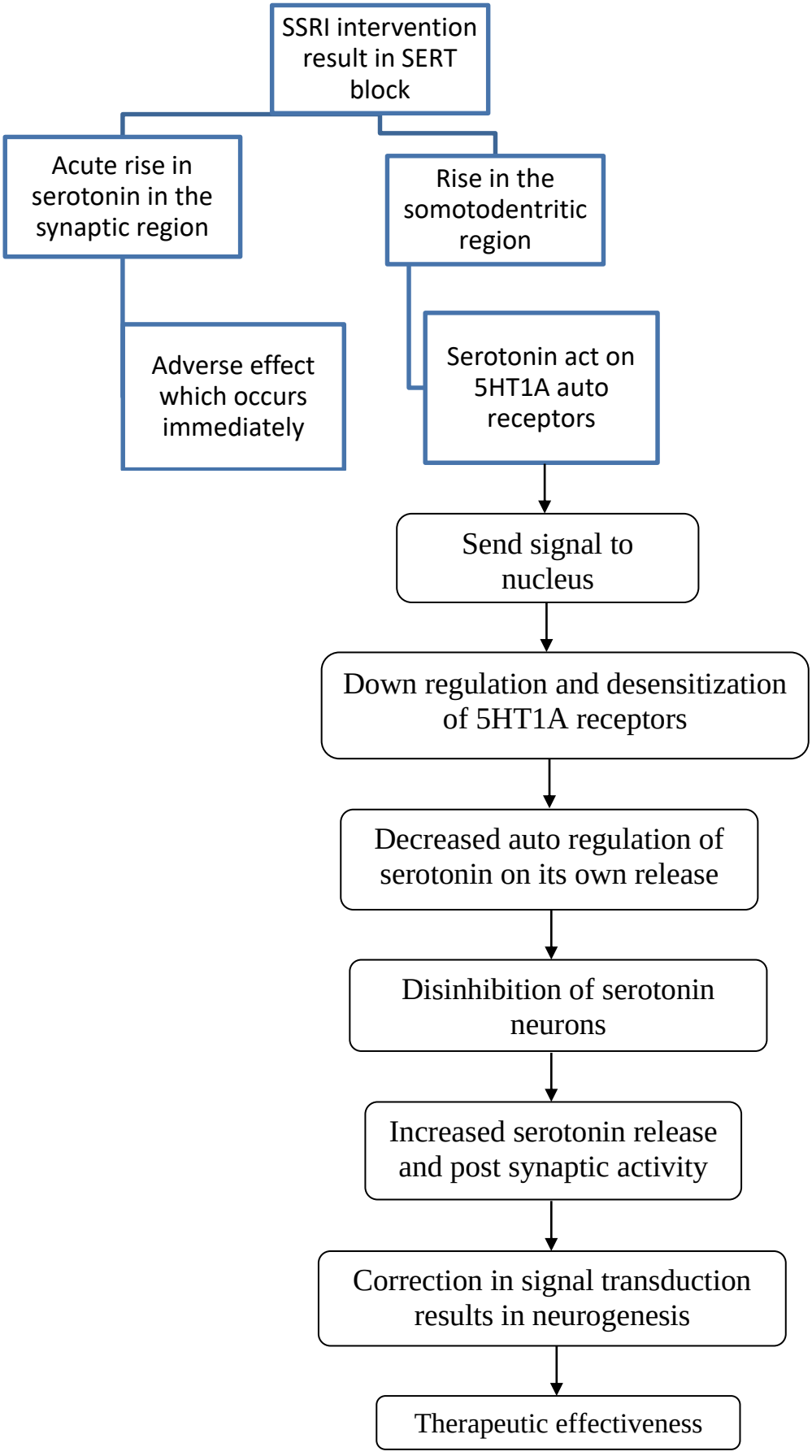
Fig 8: The primary and secondary pharmacological characters of SSRIs in common and the primary and secondary pharmacological characters of Fluoxetine

Mechanism of SSRI[28]

SSRI act by blocking the pre synaptic serotonin-auto receptor. This block will resonate in the pre synaptic somotodentritic region near cell body, resulting in increased serotonin in the region, which will provide the therapeutic effectiveness.



Cascade of therapeutic effectiveness



As in the above mentioned cascade of pharmacological events, acute rise in serotonin level in synapse immediately after the blockade of SERT is responsible for the adverse events. Increased serotonin release that happens after the desensitization of 5HT1A, due to neuronal release is responsible for its therapeutic effectiveness. The time taken for this process explains the therapeutic lag that happens with SSRI. The therapeutic effect correlates with the 5HT1A receptor desensitisation and down regulation.

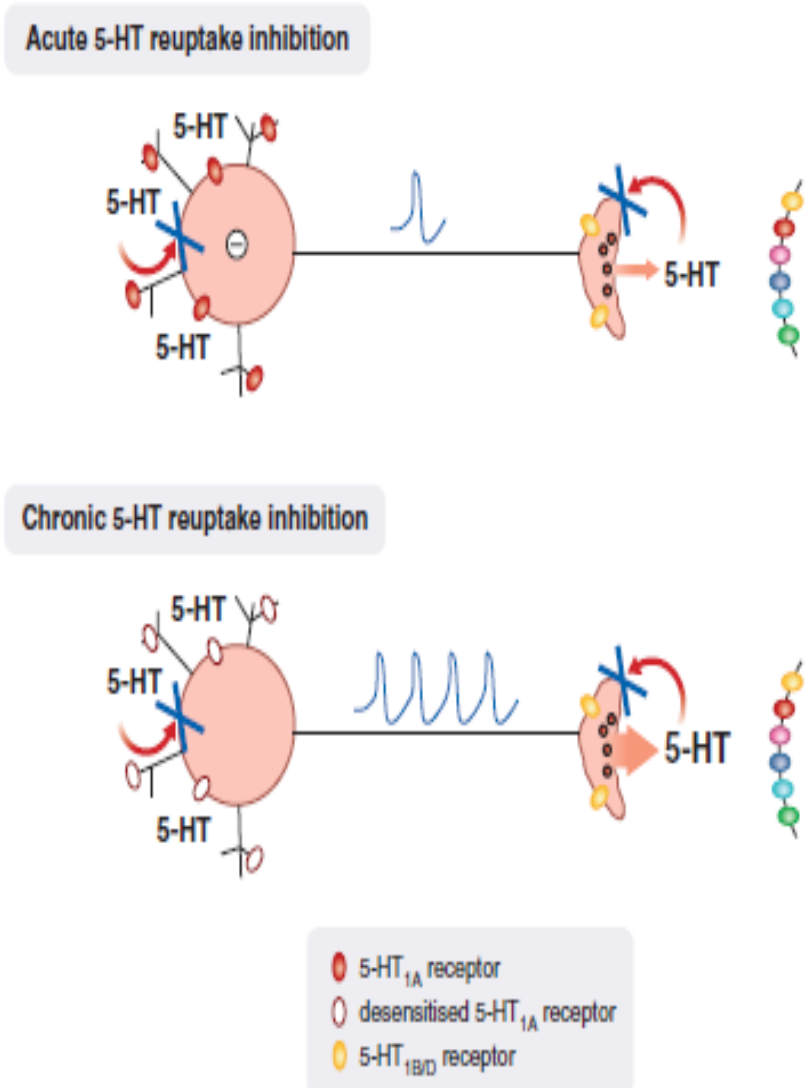


Fig 9: Effect of acute and chronic blockade of SERT on 5-HT release

Pharmacokinetics

Fluoxetine is available in oral formulation; well absorbed and bioavailability is around 70%. It is a prodrug; the active moiety is norfluoxetine. It is highly protein bound and has large volume of distribution. The elimination plasma half life of nor fluoxetine is around 180 hours which is three times more than the half life of fluoxetine. So at any given time, the plasma concentration of nor fluoxetine is always higher than that of fluoxetine. The active metabolite is the reason behind, fluoxetine being the SSRI with longest half life. The metabolites of fluoxetine are excreted through kidney.

Cytochrome p 450 (CYP) metabolism and interactions:

Fluoxetine is a substrate of CYP 2D6. It is also an inhibitor of CYP 2D6 along with CYP1A and CYP2C19 inhibition. Due to CYP inhibition it interferes with the metabolism of drugs like

- Tricyclic antidepressants
- Antipsychotics (thioridazine , haloperidol)
- Opioids (codiene, oxycodiene methadone)
- Beta blockers.

Polymorphism of CYP 2D6 among various populations has to be considered with all of the above mentioned drugs. With MAO inhibitors, fluoxetine produces a dreadful condition called serotonin syndrome. It is due to increased serotonin and is characterized by diaphoresis, tremor, autonomic

instability, cardiovascular collapse, seizure and coma. To avert this as fluoxetine has a long half life; it has to be stopped 4 to 5 weeks before starting MAO inhibitors.

Uses

As antidepressants

The therapeutic effect in depression is because of 5HT1A receptor activity. It is the commonly used drug for major depressive illness. The target dose of fluoxetine in depressive disorder is 20 mg/day, though it may be raised up to 80mg/day, many patients respond to the routine dose itself. In pregnancy the risk of untreated depression overweighs the risk to the fetus.

Other uses

- Anti bulimic profile:

  Higher dose is needed; effect is relatively quick and it is due to 5HT2C activity. They reduce the binge purge cycle. Fluoxetine is the SSRI of choice. It is arguably the most anorexigenic of the SSRIs and with a specific indication for bulimia, is also the only SSRI with direct 5-HT2C agonist activity in addition to its 5-HT reuptake blocking properties.

- Premenstrual Dysphoric Disorder

  SSRIs are effective in reducing the symptoms of PMDD. The dosing strategies with SSRIs include once daily and intermittent dosing (last two weeks of the menstrual cycle) [29]

- Obsessive Compulsive Disorder.(OCD)

In addition to CBT which has efficacy per se of 50-70%, SSRIs when added can prevent the relapse in OCD.

- Generalized anxiety disorder and panic social anxiety disorder

Similar to therapeutic lag in anti depressant effect, SSRIs take two to three weeks to act in this disorder. The dose is 5–10 mg/day.

- Post Traumatic Stress Disorder:

There is no complete recovery with SSRI, but it decreases the symptoms and provides functional improvement. In responders, the period of treatment is 6months to one year in acute episodes and one to two years in chronic episode.

ADVERSE EFFECTS

The adverse effects are less than TCAs, because of selective SSRI action.

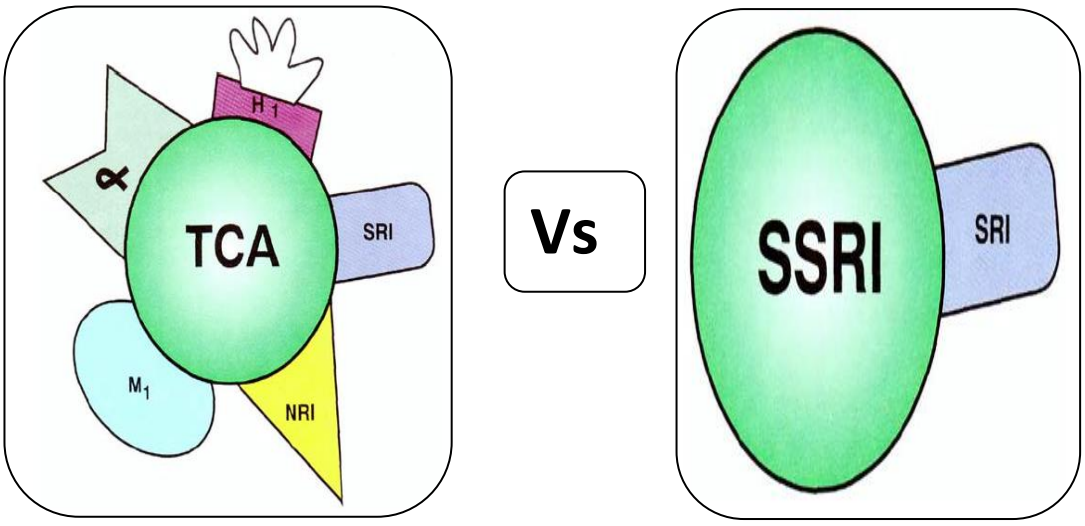


Fig 10: Illustrating the receptor specificity of SSRI

SSRIs increase serotonin level in both desirable and undesirable pathway. Serotonin action in the undesirable pathway and receptor is responsible for its adverse reaction. With SSRI the adverse effects happens faster than the therapeutic effect, as it is due increase in post synaptic serotonin instantly after SERT blockade.

The common adverse effects are

Gastrointestinal complaints

CNS disturbances

Sexual dysfunction.

- **Gastrointestinal complaints**

Within 1 -2 hours of ingestion they induce gut irritation and produce nausea which is a common complaint. So it is advised to be taken with food. Nausea in SSRI may also be due to stimulation of 5-HT3C receptors in the chemoreceptor trigger zone (CTZ). CTZ mediated nausea needs dosage titration. Incidence of diarrhea is around15-20%.

- **CNS disturbances**

The frequent symptom is sleep disturbance; fluoxetine is the most common SSRI producing this symptom. So it should be taken at morning after breakfast. Other side effects are sedation, numbness and in few cases extrapyramidal side effects.

- **Sexual dysfunction.**

Incidence of sexual dysfunction is around 30% to 50%; more common among men, but in severity it is worse in females. The major complaint is delayed orgasm, though it happens with all SSRI more common with paroxetine and fluoxetine [30].

- **Other adverse events**

Dry mouth and headache occur with SSRI and, although rare some somatic complaints like bruxism, sweating, few incidence of dilutional hyponatremia are prevalent in the general population and on chronic use it produces weight gain.

Withdrawal

Because of long half life there is no need for slow tapering as with other SSRIs.

Other Antidepressants

- **Tricyclic antidepressants**

Before SSRIs, Tricyclic antidepressants were the commonly used drugs. Though therapeutic efficacy is similar to SSRIs, they are drugs having narrow therapeutic index so not preferred over SSRIs. The acute toxicities of TCAs are hypotension, respiratory depression, cardio toxicities like prolonged QT and broad QRS .The usual adverse effects are sedation, hypotension, weight gain,

sexual dysfunction, convulsion, anticholinergic effects, and hypersensitive reaction. Other than depression it is used in wide range of disorders like OCD, anxiety, phobias, attention deficit hyperactive disorder, nocturnal enuresis, migraine, psychosomatic disorder.

Serotonin norepinephrine reuptake inhibitors (SNRIS)

SNRIs are chemically different from each other. They block both Serotonin and Nor epinephrine transporters, similar in efficacy when compared with SSRIs, but with short half life. In adverse effects they are very similar to SSRIs. They exhibit dose dependent increase in efficacy due to weak Norepinephrine Reuptake Inhibition. Adverse effects are due to augmented adrenoceptors activation; they are insomnia, sexual dysfunction, headache, dry mouth, dizziness, sweating and loss of appetite.

Noradrenaline uptake inhibitors

Reboxetine and atomoxetine are the drugs that are highly selective for noradrenaline. Their efficacy is less than the TCAs. Atomoxetine is used in attention deficit hyperactivity disorder.

5 HT receptor modulators

Trazodone and nefazodone are the drugs. The activity is due to 5-HT2A receptor blockade. Nefazodone is also a weak inhibitor of both SERT and Nor epinephrine transporter (NET). Trazodone has an additional α blocking property.

Monoamine oxidase inhibitors

First introduced antidepressant, but it is now used only in resistant cases. Non specific MAOIs are not used now because of adverse events associated with drug and food interaction. Specific inhibitors are introduced. MAO-B inhibitors do not have any antidepressants effect at doses that are precise for MAO-B inhibition. New class of agents- RIMA like Moclobemide is now used as antidepressants.

Melatonin agonist

The drug is Agomelatine**.** It act as an agonist at receptors MT1 and MT2 .It has a short plasma half-life. It is used in severe depression; it works by regulating the circadian rhythm which is often disturbed in depression.

CHOICE OF ANTIDEPRESSANTS

Most of the antidepressants are equal in efficacy against Major Depressive Disorder, so it is tough to pinpoint one antidepressant to be consistently better than the other. Thus, the choice will be based on cost, adverse effects, availability, patient's response to therapy, drug interactions, age, and medical issues.

SSRIs are the preferred choice due to their efficacy, safety, tolerability profile. There is no need for dose titration. Other drugs like SNRIs, mirtazapine, bupropion also provide a choice as first-line management for MDD.

Individual drug of choice for a patient is going to be tailor made for that particular patient. If the patient complaints of insomnia and sexual dysfunction due to SSRIs then mirtazapine serves as a choice as it promote sedation and no sexual dysfunction. In elderly due to anticholenergic side effects TCAs are not commonly preferred. Moreover TCAs and MAOIs are now regarded as second and third choice in the management of MDD.

COURSE OF TREATMENT

The purposes of the treatment is to cutback the acute symptoms, aid the patient to return to functioning life and to prevent consequent episodes. The course of the disease used to be, single cycle of MDD if untreated last for 6–14 months, in few patients it may last even longer. Once pharmacotherapy is initiated it will take 3 to 4 weeks for the drug to act. So for any antidepressant we have to give a trial period of 8 -12 weeks, before judging the therapeutic efficacy. This therapeutic lag of antidepressants is due to the time taken to adaptive changes in the neuronal plasticity that happens secondary to increase in synaptic neurotransmission. The objectives of the treatment are[31]

1) Remission –decrease in symptoms
2) The continuation phase for 4 to 9 months has to be given in the post remission period. The target of this phase is to get rid of the residual symptoms and prevention of relapse and
3) The maintenance phase is for minimum of 12 to 36 months in which the target is to counter reoccurrence.

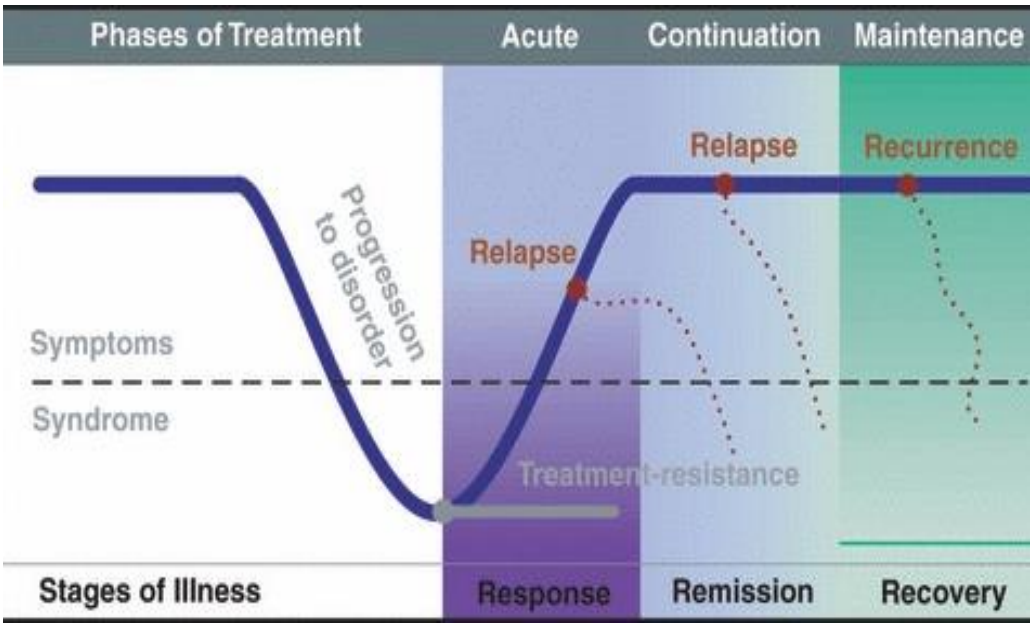


Fig 11: Course of depression

Remission - Absence of symptoms

Relapse - Return of symptoms not over a period of 6 months of remission

Recurrence - A separate episode of depression

The span of antidepressant therapy depends upon the risk of recurrence. The hazard of recurrence increases proportionate to the number of previous episodes. For persons who are at increased risk for reoccurrence, such as

i) Patient less than 40 years with two or further previous episodes and

ii) Irrespective of age with three or further previous episodes

Investigators recommend lifelong maintenance therapy.

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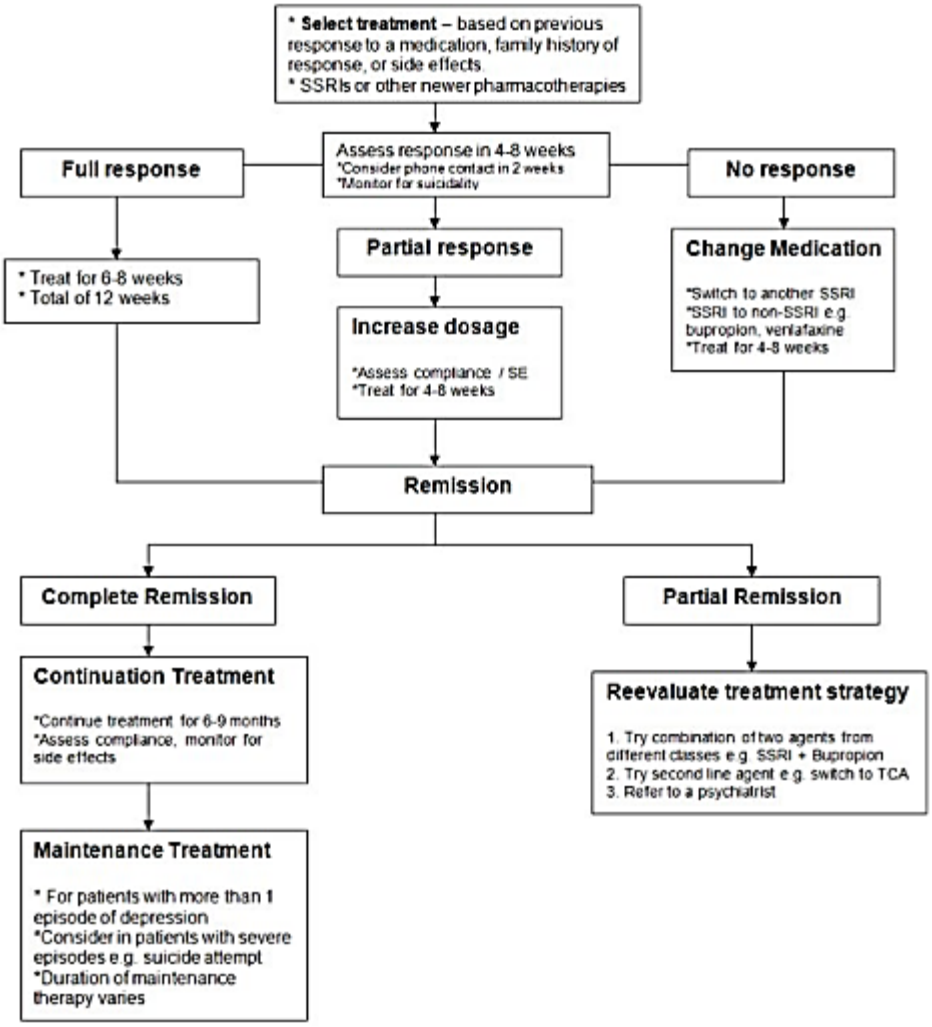


Fig 12: Algorithm of MDD treatment

Lag in antidepressant effect and the importance of compliance should be well informed to the patient, family members and friends.

Newer targets

Vilazodone and vortioxetine, are recently marketed drugs for MDD, they target both 5-HT1A receptors and the SERT in their action. Thus they refine and expand the monoaminergic mechanisms[32].

In addition based on various biological hallmarks of the disease and efficacy of pharmacological interventions, different hypotheses of depression are being put forward in translational and clinical researches. These targets are beyond SSRIs and SNRIs. They are multitarget or multimodal approaches that have additive or synergistic effects on selected biological pathway. Some examples are

- Neurokinin system-particularly Nk_1 antagonists.

  Neurotensin

  Chole-cystokinin,

- Cannabinoid system

  Anandamide

- Glutamate system

  NMDA receptor antagonist

  AMPA modulators

- Opioid syatem

  κ opioid receptor Antagonists

- Cognitive enhancers

  Ampakines

  Nicotinic cholinergic receptor

  Erythropoietin

- GABA system

  GABA B antagonist.

- Others

  GRII cortisol receptor antagonists,

  Melanocyte inhibiting factor (MIF-1) analogues

- **Miscellaneous agents**

  Methylfolate,

  Oestrogen

  Omega-3 Fatty Acids

  S-Adenosyl Methionine

  Folic Acid

- **Plant derived drugs**

  Hyperforin,

  Kava,

  Ginkgo biloba,

  Curcuma longa,

  Rosmarinus officinalis

STATINS

Statins are commonly used cholesterol lowering agent. They are the most effective drug for dyslipidemia. The precursor of stain was biosynthesised from Penicillium citrinium. Mevastatin was the first Statin with demonstrable therapeutic potential. Then from Aspergillus terreus, lovastatin was derived[33].

Now the available statins are

Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin.

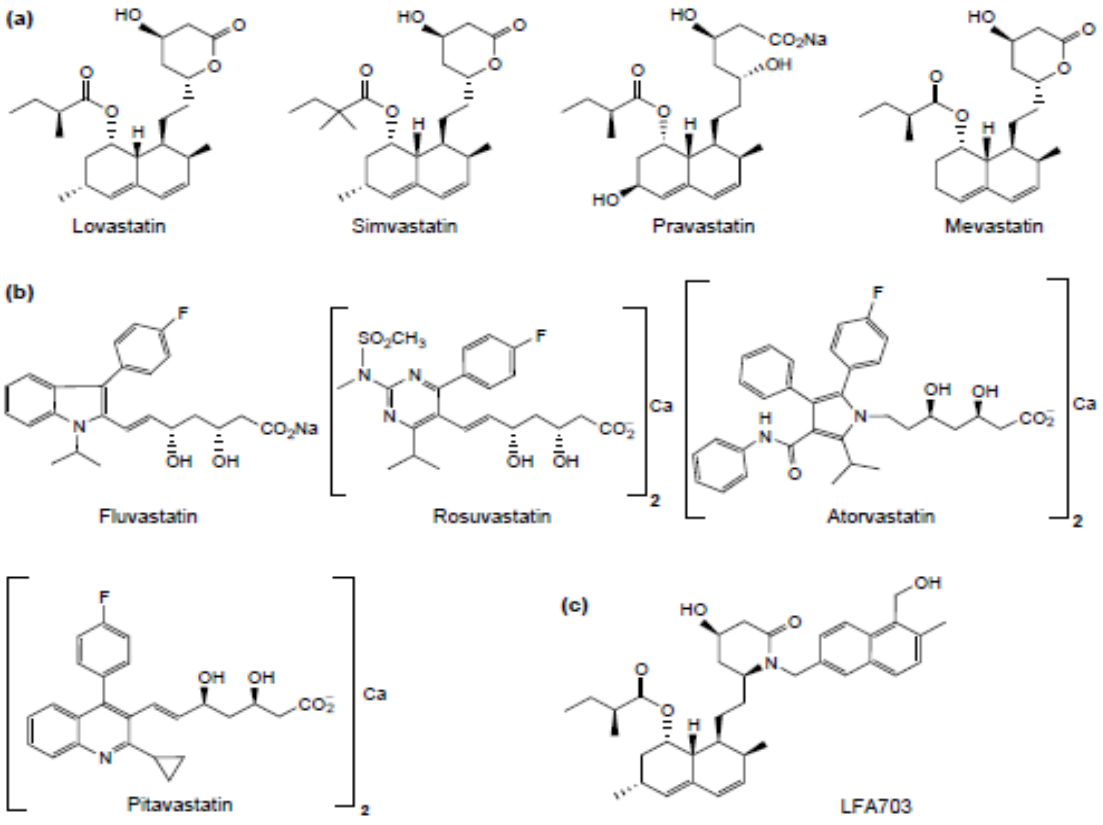


Fig 13: Structure of Statins

Simvastatin and pravastain are derived from lovastatin. The synthetic ones are atrovastatin, rosuvastatin, fluvastatin and pitavastatin. All the synthetic compounds contain fluorine.

Based on their potency of Low Density Lipoprotein (LDL) lowering effect Statins in descending order

Pitavastatin

Rosuvastatin

Atorvastatin

Lovastatin

Pravastatin

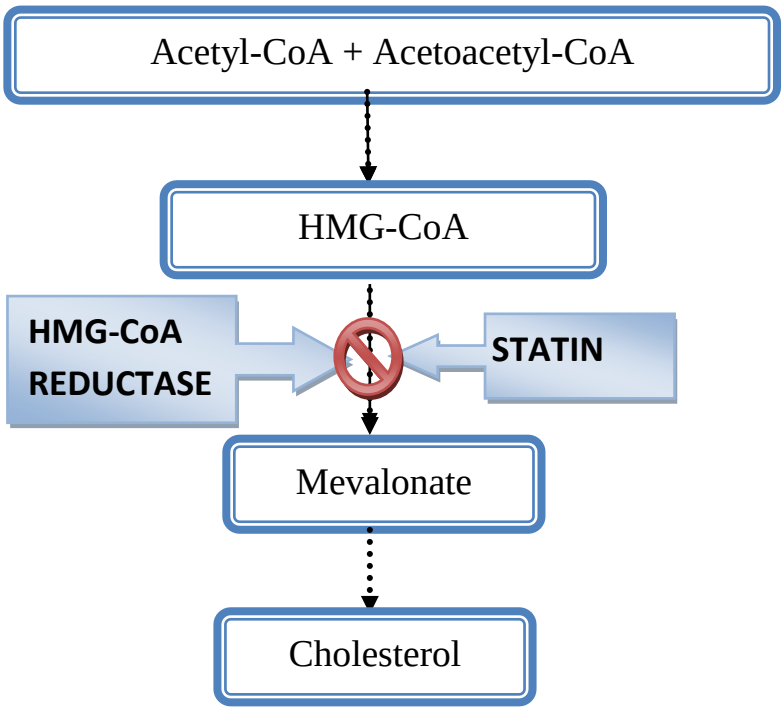
Simvastatin

Fluvastatin .

The potency of Statins is dose dependent[34].

Mechanism of action

The mechanism of action of Statins is due to the inhibition of the enzyme 3-Hydroxy- 3 Methyl Glutaryl Coenzyme A. (HMG CO-A) Reductase. This enzyme is involved with cholesterol biosynthesis. Statins act as a competitive inhibitor of this enzyme. It decreases the biosynthesis of cholesterol[35].



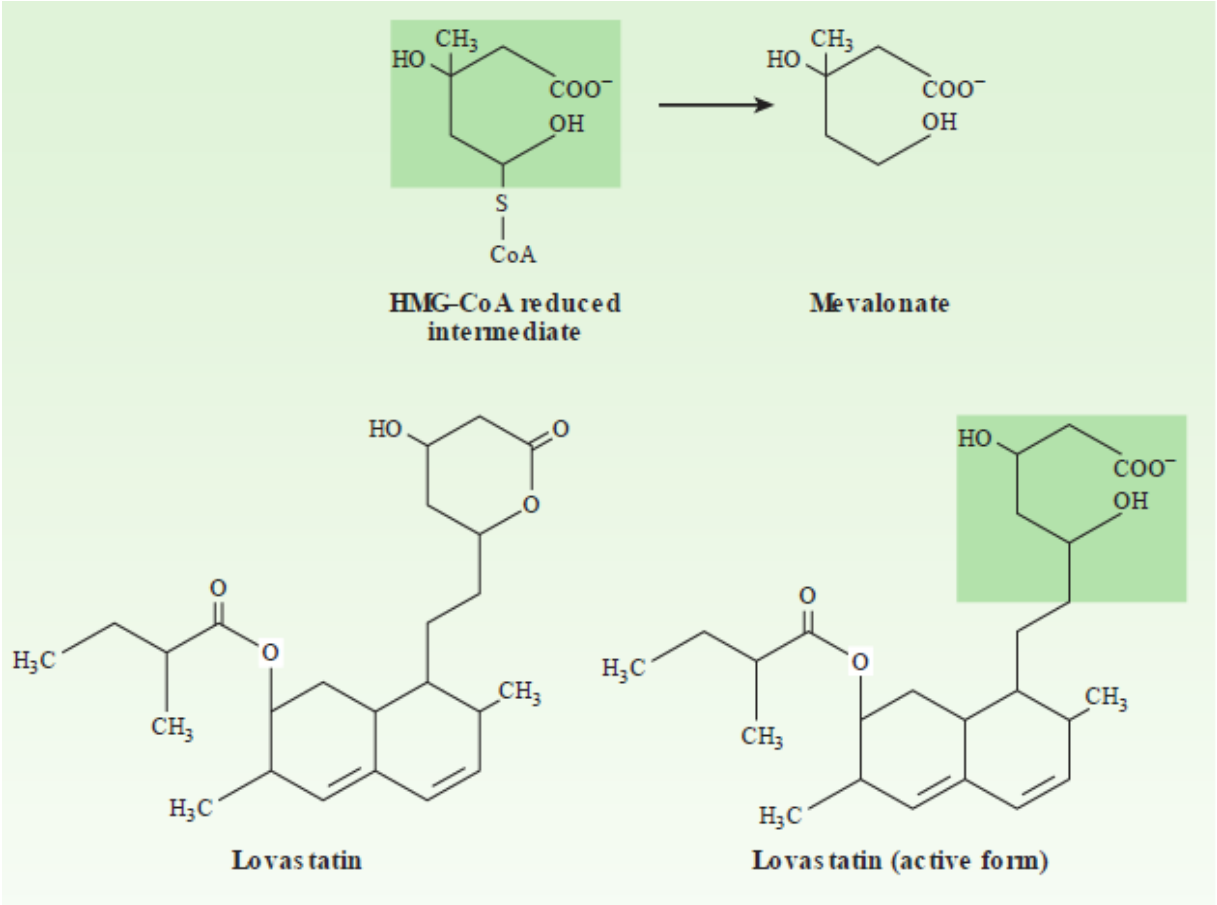
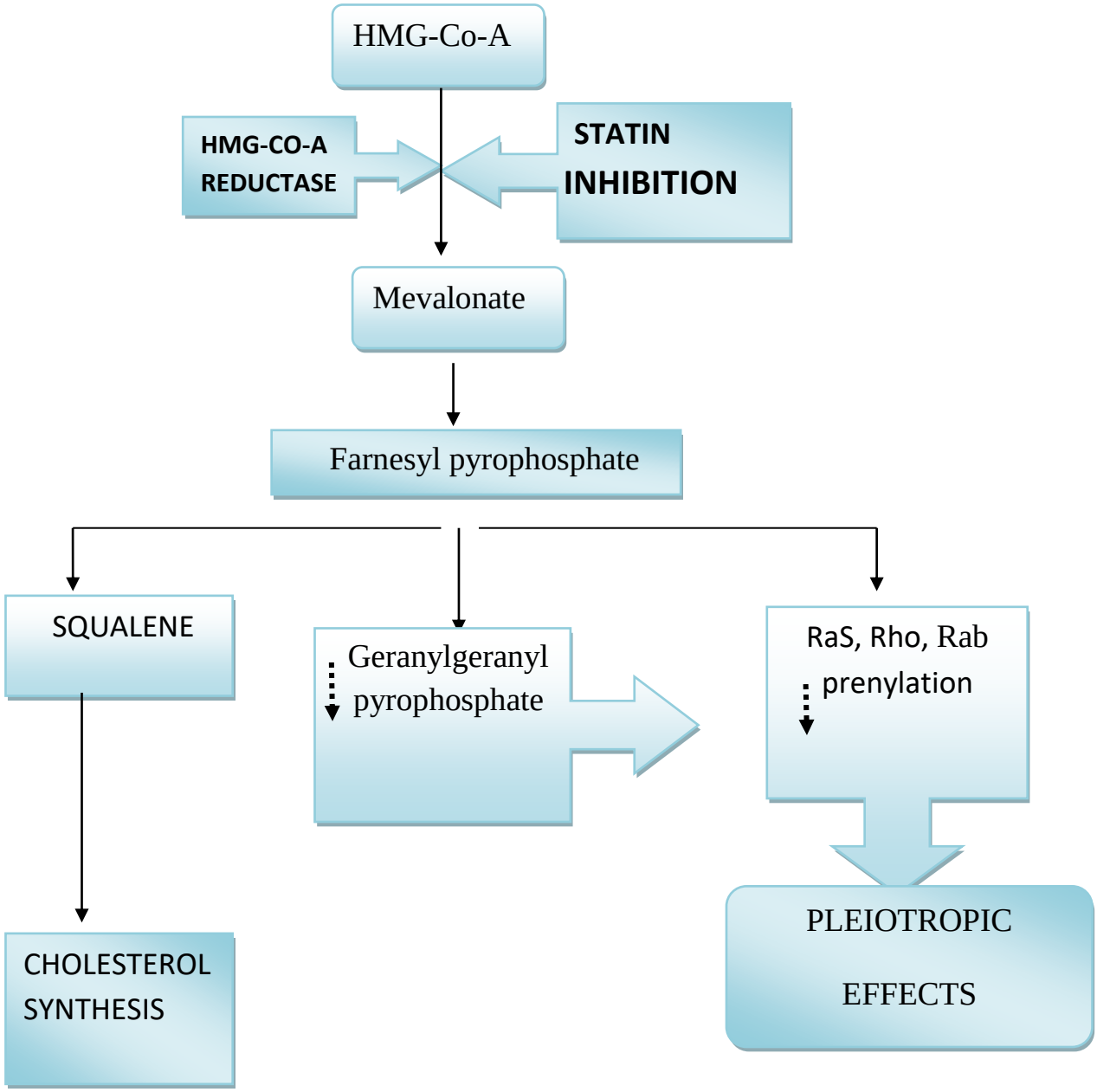


Fig 14: HMGCo-A has structural similarity to active form of Lovastatin

The fascinating thing in the cholesterol synthesis is the intermediate substances like ubiquinone and dolichol, which are produced in this cycle in addition to cholesterol. These intermediates are isoprenoids. They are involved in a reaction called Prenylation. It is a lipidic modification of cellular proteins during the post translational period.



Farnesyl and Geranylgeranyl isoprenoids which are adjuvant products of the cholesterol synthesis pathway irreversibly bind themselves to Ras and Ras-associated GTP-binding proteins, Rho proteins and Rab proteins and prenylate them[36].

Competitive inhibition of HMG-CoA reductase by statins not only decrease cholesterol synthesis but also decreases these isoprenoids that are required for prenylation. A study by Bassam R. Ali et.al[37] demonstrated the reversible inhibition of prenylation of signaling proteins by Mevastatin. This supplementary mechanism of statins are accountable for their various pleiotropic effects

Statins have the following pleiotropic effects[38]

- Better endothelial activity
- Decreases the vascular inflammation
- Improved ischemic tissue neovascularisation
- Reduced platelet aggregation
- Atherosclerotic plaque stabilization
- Increased endothelial progenitor cells
- Increased fibrinolysis
- Antithrombotic activity
- Immunosuppressant

- Protection against infection
- Inhibition of migration of germ cell during embryogenesis.
- Anti cancer property

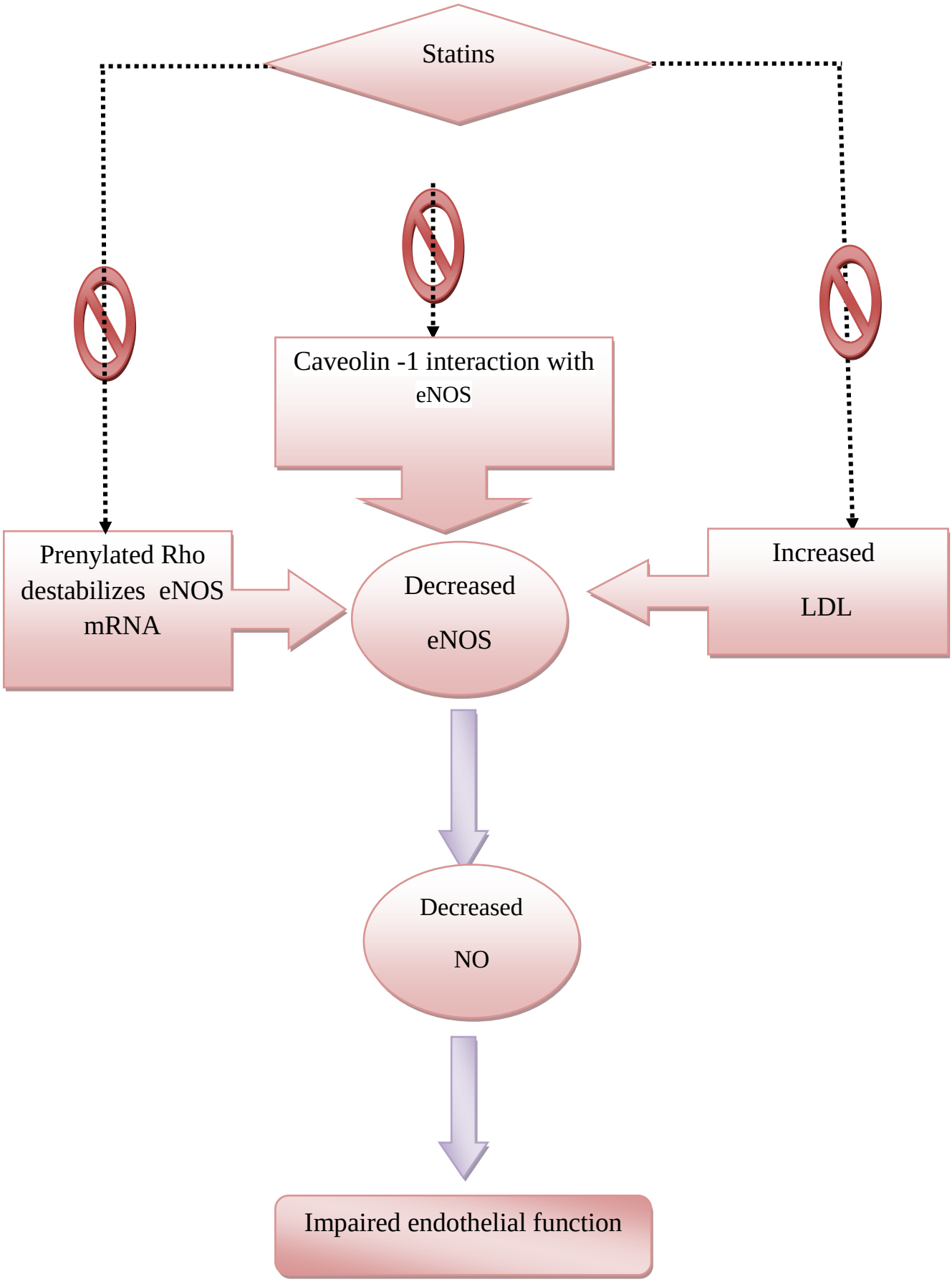
Neovascularisation

- Statins increase the endothelial progenitor cells[39].
- Increase their migratory capacity
- Alter their adhesiveness and
- Enhance their mobility towards injured site thereby augment the neovascularisation of ischemic tissue.

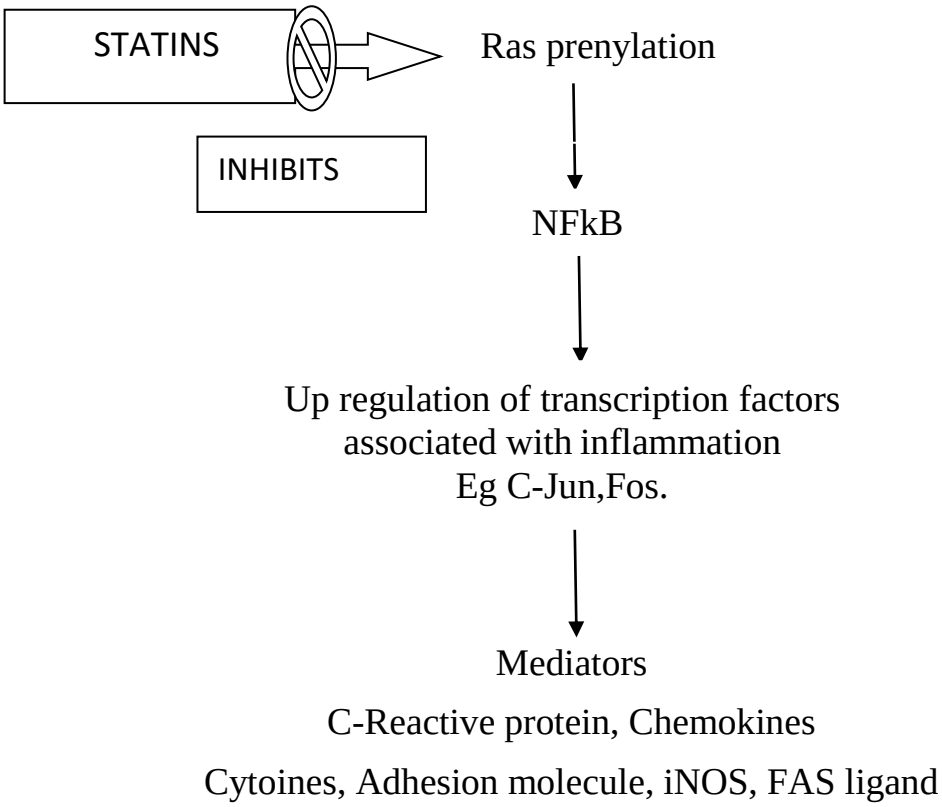
Effect on Endothelium

In hypercholesterolemia the endothelium mediated vasodilatation is affected. This is due to impaired nitric oxide (NO) production. Statins increase the NO production by the endothelium. Statins induced NO synthesis is by

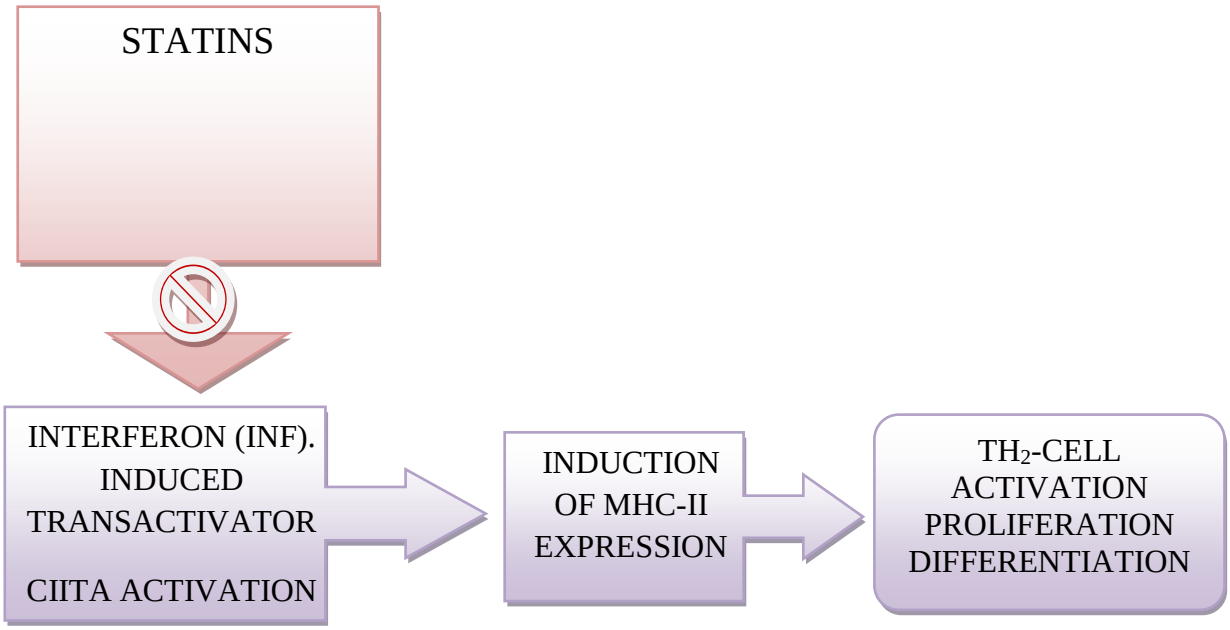
1) blockade of LDL induced down regulation of endothelial Nitric Oxide Synthase (eNOS)
2) Mechanism irrespective of its cholesterol Lowering effect.
    - Statins suppress the Rho/Rho signaling activity which decreases the eNOS mRNA stability
    - It decreases caveolin-1 expression in endothelium, which disintegrates the eNOs and hence increases NO.


Statins
Caveolin -1 interaction with eNOS
Prenylated Rho destabilizes eNOS mRNA
Decreased eNOS
Increased LDL
Decreased NO
Impaired endothelial function

Anti Inflammatory Effect



Statins as Immuomodulator[40]



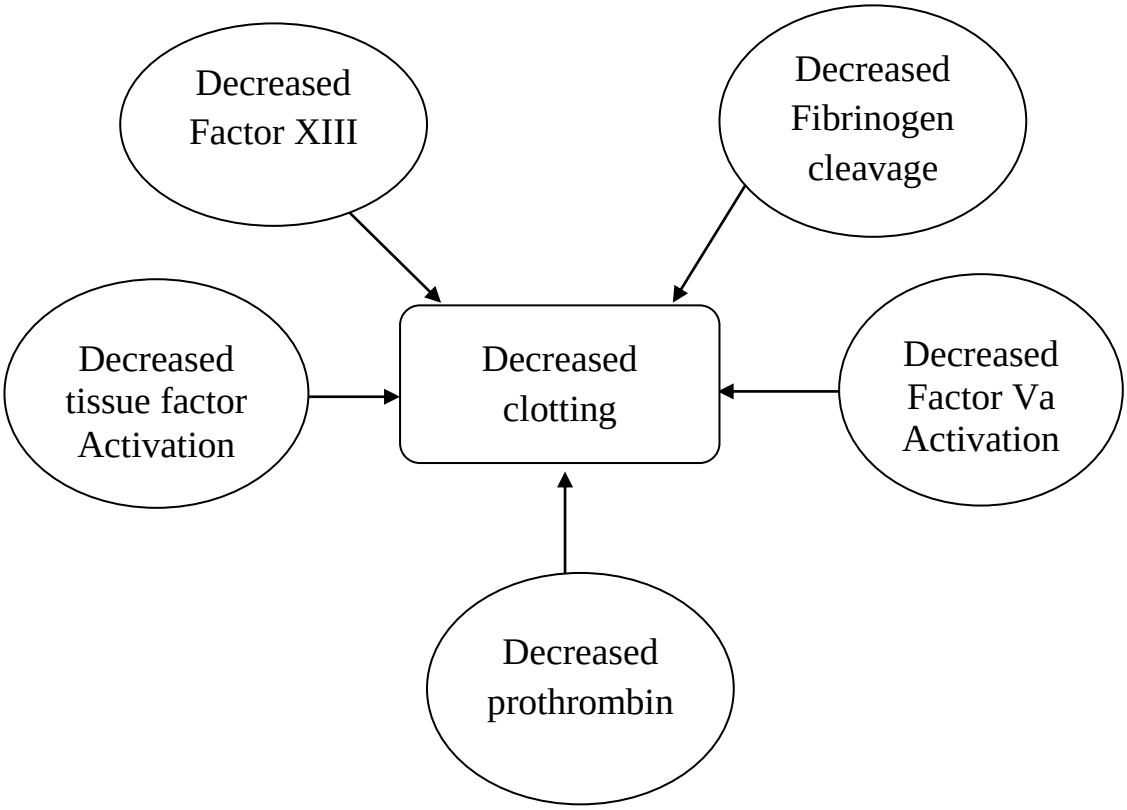
Effect on Plaque Stability[41]

Statins ensure plaque stability by two mechanisms:

i) Reduction in macrophage accumulation at plaque site.

ii) Reduction in Matrix metalloproteinase (MMP) secretion thereby reducing the plaque rupture.

Anti thrombotic activity[42]**.**



Effect on Lipoprotein Oxidation

Oxidized LDL is proinflammatory. Anti-oxidant activity of statin is due to

- Decreased Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[43]
- Decreaed superoxide formation

- Decreased oxygen free radical
- Neutrophil oxidative burst

Effect on Fibrosis

Statins can decrease fibrosis by inhibiting the angiotensin-mediated growth factor production for the connective tissue growth. MMP expression is also reduced.

Anti Cancer Property [44]

In the cell cycle, statins prevent the progression of G1-S Phase resulting in anti proliferative effect. Experiments showed that this anti cancer effect is more attributed to free geranylgeraniol rather than farnesylated proteins inhibition.

Other Effects:

Statins increase the bone growth factor- Bone morphogenic protein (BMP)-2. Hence preventing osteoporotic changes in animal model[45]. They have antiepileptic activity by blocking the NMDA pathway

Pharmacokinetics of Statins [46]

Lovastatin and Simvastatin are prodrugs. All other statins are active drugs. The above mentioned prodrugs are administered as lactones. Their active metabolite is beta -hydroxy acid. Pravastatin, Fluvastatin are sodium salts; Atrovastatin is a calcium salt of active beta hydroxy acid.

They are fat soluble substances; have high absorption when combined with food except pravastatin. These substances have enormous first pass metabolism, so the systemic availability is around 5-20%. Cholesterol lowering effect happens within one to two weeks of therapy. Brain is the cholesterol rich organ in the body. Statins have high CNS permeability. The hypolipidaemic effect of Statins would not alter the cell membrane's cholesterol homeostasis in the central nervous system. Atrovastatin and rosuvastatin are long acting; all other Statins have half life around 1-4 hours. Atrovastatin metabolites are active inhibitors of HMG Co-A reductase, all statins are excreted by liver.

Adverse Effects[47]:

- **Myopathy:**

Myopathy is a clinically significant adverse effect of statins. The incidence is very low and it is around 0.1% to 1%. It happens with all the statins. Creatine phosphokinase (CPK) monitoring is warranted when patient complaints of muscle weakness, soreness. When myopathy happen it settles on withdrawl of the drug, we can rechallange with other statins.

- **Hepatotoxicity:**

It is dose related and characterised by increase in transaminase enzyme. The incidence is inconsistent, and very rare at therapeutic doses. So the protocol is to have a base line value of alanine aminotransferase, then once in three

months in the initial period and once in a year or every six months thereafter. Incidence increases in patient with underlying liver pathology.

Other minor adverse reactions that fade out on chronic usage are headache, gastrointestinal disturbance, peripheral neuropathy, lupus like syndrome.

Drug interaction and Statins

Lovastatin, Simvastatin, and Atrovastatin are substrate of CYP3A4. Fluvastatin, Rosuvastatin are substrate of CYP2C9. Enzyme inducers will reduce the plasma concentration of statins and enzyme inhibitors like antifungals, macrolide**,** antibiotics, protease inhibitors, nefazodone like antidepressants increase the concentration of statins thereby the risk of myopathy.

Statins are contraindicated in pregnancy, because their effect on foetal development is uncertain[48]. It is safe in children above 8 years of age.

Therapeutic Use

- Common therapeutic indication of statins is dyslipidaemia for primary and secondary prevention of coronary events.

Due to its pleiotropic effect it is used in

- Cardio Vascular Diseases (CVD)
    - Statins decrease the risk of cardio vascular diseases in patient with metabolic syndrome

- it decreases the incidence of CVD in apparently normal old people irrespective of hyperlipidemia and metabolic syndrome[49]
- In primary hypertension in children, the use of statin decreases the incidence of atrial fibrillation probably due to its anti inflammatory property[50].

- Preoperatively used in coronary revascularization surgery.

  Percutaneous coronary intervention is associated with myonecrosis due to platelet aggregation and distal embolisation in response to inflammation. Statin decrease it.

- Irrespective of the cholesterol level it is used in patient of >40 years of age with type II diabetes[51]
- In thrombo ischemic attack and stroke, statins decrease the risk of recurrence [52]
- Renal artery stenosis & Acute limb ischemia

Statins and Depression

Statins and depression has to be viewed in the context of

- Cholesterol level and depression
- Pleiotropic effect of statins and depression

Cholesterol level and Depression

Though the literature is flooded with information about the relationship between cholesterol and depression, it is still elusive. The relationship between

cholesterol and depression is two faceted. Some studies demonstrate that lower blood cholesterol level is associated with depression. Series of case reports are published about statins being associated with cognitive impairment, the rationale provided were,

- Low esterification of cholesterol is associated with alterations in the composition of cholesterol and phospholipids on the cell membrane. It results in altered receptor density, neurotransmitter exocytosis, and signal transduction.
- There is association between, low level ω_3 fatty acid and increased ω_6 / ω_3 ratio with depression[53].
- Cholesterol is essential for the synthesis of neurosteroids. Long term exposure of statins to the neurons of the brain endangers the formation of synapses; particularly in the hippocampus which predisposes to major depressive illness.

Metanalysis has proved statins are not associated with any cognitive impairment[54]. Many studies show that there is no correlation between lower cholesterol level by statins and depression. Even some studies proved that high cholesterol levels are associated with treatment resistant depression. Also it needs mention that the use of statins for cardiovascular diseases like myocardial infarction (MI) showed decreased scores of post MI depression in those patients.

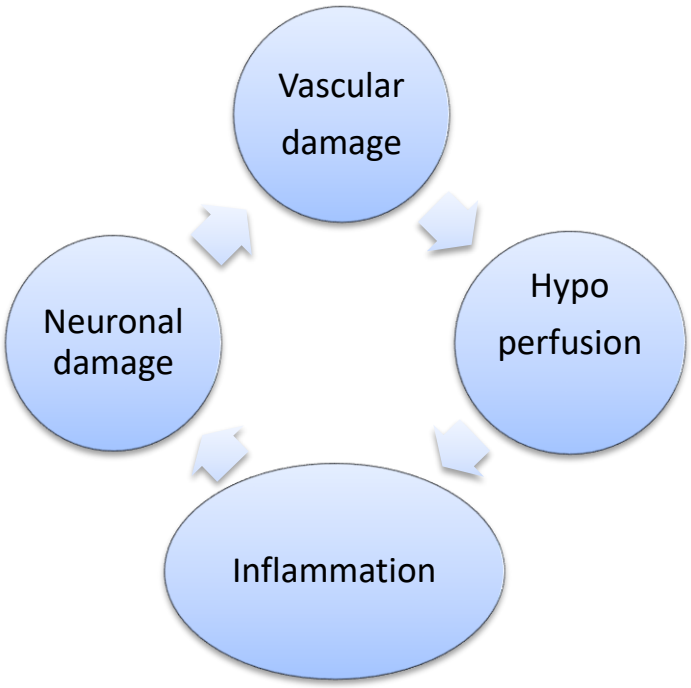
Pleiotropic Effect of Statins and Depression

Inflammation, free radical injury and vascular pathology are the etiological factors for depression. Pleiotropic effect of Statins combat all this.

Vascular depression and statin

Vascular depression is most common in geriatric age. Any pathology in the brain vasculature can predispose as well as precipitate depression.

Vascular depression is characterised by white matter hyper Intense lesion on imaging. It is quite resistant to treatment with conventional anti depressants



In this study statins were evaluated for its anti depressant property with fluoxetine as standard by tail suspension method.

Evaluation Methods for Antidepressants[55]

Most of the antidepressant drugs that we use today are generated based on the monoamine hypothesis. The foremost one is non-selective MAOIs. They are coupled with dangerous food and drug interactions. With TCAs the disadvantage is its additional alpha receptor blocking and narrow therapeutic index which produce life threatening toxicities. To overcome these drawbacks, SSRIs were introduced. They are better tolerable and safer than TCAs. They have side effects like insomnia and sexual dysfunction which makes the drug less compliant. There is always a search for antidepressant which are safe and efficacious and with fewer adverse effects.

In evaluating anti depressant activity through animal studies, the major downside is that the animal models cannot simulate major depressive illness as in human beings. So the principle behind the various screening models is comparing the new drug with already standardized anti depressant. There must be a correlation between the standardized antidepressant and the effect of test drug on the animal model.

In vivo methods in animal are:

1. Amphetamine potentiation
2. Apomorphine antagonism
3. Chronic social defeat stress
4. Forced swim test
5. Isolation induced hyperactivity

6. Learned helplessness test
7. Muricidal behavior in rats
8. Olfactory bulbectomy
9. Postpartum depression models
10. Reserpine induced hypothermia
11. Resident intruder paradigms in rat
12. Tail suspension test
13. Water wheel model

Of the above mentioned test, tail suspension method is based on the principle of behaviour despair model which was used in this study. Other test that are based on the same principle are

Water wheel model

Forced swim test

WATER WHEEL MODEL

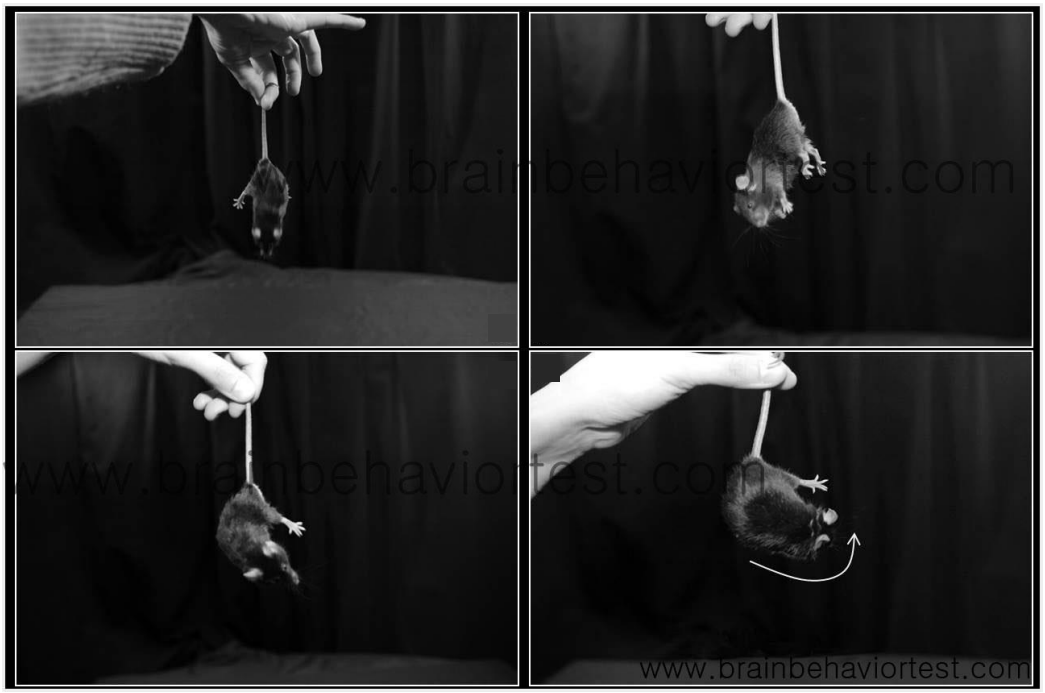
It is based on the principle 'behavioural despair activity'. Animal is forced to swim in a tank filled with water. A rotating wheel presents inside the tank fake itself as an option for escape. The animal tries it and due to their weight it again turns them to water. The animal becomes desperate and immobile when depressed. The antidepressants increase the number of attempts of escape; measured by number of rotation of the wheel.

FORCED SWIM TEST

In a restricted water tank, mice or rats are enforced to swim, with nil probability to escape. After an initial span of struggling, the animal develops a characteristic behaviour of immobility. Duration of immobility gets reduced with antidepressants.

TAIL SUSPENSION TEST

Tail suspension test is based on the principle of behavioral despair. It was first done by Steru et al. Reproducibility is more in this experiment if we use mice rather than rat. It is a very reliable and rapid method used in screening new antidepressants. When mouse is suspended in the box with its tail plastered with an adhesive tape, it cannot escape or hold on to nearby wall, 'behavioral despair'



is induced. The test is done for a period of 6 minutes. The mice will exhibit escape-oriented behavior. Due to desperation mice will be immobile for a while. The duration of immobility is noted for the whole 6 minutes. The immobility period is directly proportional to the degree of depression. This test is not suitable for MAO inhibitors. These animal models are best for drugs which are using serotonergic mechanism. This test can also be done using a computer assisted system. By this we can calculate the immobility, and activity of the mice in real time.

MATERIALS AND METHODS

MATERIALS AND METHODS

The current study, evaluated the anti- depressant activity of the pharmacological agent statin in adult male Swiss albino mice by tail suspension method. For the study, approval was obtained from Institutional Animal Ethical Committee of Madurai Medical College, Madurai. Dated 23.03.2017.

Study Centre

Institute of pharmacology, Madurai medical college, Madurai.

DURATION

Study period was 6 months since March 2017.

NUMBER OF ANIMALS USED

30 adult male albino mice of weight 25 -30 grams.

DRUGS REQUIRED

Standard group was treated with Capsule. Fluoxetine 20mg /kg

Test groups were treated with various statins.

Test I with Tablet Rosuvastatin 10 mg/kg

Test II with Tablet Atrovastatin 10 mg/kg

Test III with Tablet Simvastatin 10 mg/ kg

Control group received Distilled water

Other materials required

Weighing machine

Stirrers and beakers

2ml syringes

Stainless steel oral feeding tube

Tail suspension box

Plastic tubes 4 cms x1.5cms as climb stoppers

Adhesive tapes

Stop watch

Digital camera

Drugs

Rosuvastatin 10mg, Atrovastatin 10 mg, Simvastatin 10mg tablets were used for test group I, II, and III respectively and at a dosage of 10mg/kg. They were dissolved in distilled water and given orally. Capsule. Fluoxetine was used on the standard group at a dosage of 20mg/kg, dissolved in distilled water and given by oral route.

Procedure

30 male Swiss albino mice were procured from our central animal house, Madurai Medical College, Madurai. The animals were sheltered in a cage made of polypropylene and they were maintained at room temperature with a12hours:12hours light/dark cycle. They were provided with free access to food

and water throughout the day, and they had it ad libitum. The experiment was conducted at their domicile itself.

The principles of committee for the purpose of controlled supervision of experimentation on animals (CPCSEA) guidelines were followed since procurement to the follow up period, and throughout the study period the animals were handled by well trained personnel, with adequate care.

The 30 animals were divided into 5 groups of 6 animals in each group. Group I served as control, group II served as standard, group III, group IV, group V served as test group. Drugs were administered orally, for a period of 14 days once daily in the morning as mentioned in the table below according to the group.

Drug details of each category

Group	Study group	TREATMENT
I	CONTROL	Normal feed and Water+ Distilled water
II	STANDARD	Normal feed and Water + Cap. Fluoxetine 20 mg/kg orally
III	TEST -1	Normal feed and Water + Tab. Rosuvastatin 10 mg/kg orally
IV	TEST-2	Normal feed and Water + Tab. Atrovastatin 10 mg/kg orally
V	TEST-3	Normal feed and Water + Tab. Simvastatin 10 mg/kg orally

ORAL FEEDING TECHNIQUE

Feeding tube used was a hypodermic needle made of stainless steel. It has a blunted tip, which is soldered with a small ball around it. A two ml syringe which contained the drug to be administered was attached to the needle. Mouse was gently grasped by the nape of neck , and secured in the left hand. The oral feeding tube was introduced in the inter dental space laterally. Then with a gentle rotatory movement it was advanced into the oesophagus. On reaching the desired level, the drug was pushed gently.

Tail suspension test

Tail suspension test was performed on day 7 and day 14 of the experiment, one hour after the drug administration. It was carried out for all the groups in the experiment such as the control, standard, test –I, test –II, test III in such a way that all the six animals belonging to a group were tested simultaneously.

The tail suspension box is a rectangular chamber made of plywood painted in brown colour to provide the contrast. The dimensions of the box were height 55 cm width 90cm and depth 11.5 cm. It was divided in to six chambers by placing dividers, so that each chamber had a width of 15cm. There was a provision in the roof of each compartment to hang the mice. The space in each compartment was adequate to prevent the mouse from getting into contact with the wall. The distance between the floor and the tip of nose of the suspended mouse was around 20-25cms. A detachable tray was placed in the bottom of each compartment to collect the excreta.

Clear hollow cylindrical plastic tubes of dimension 4 cms x 1.5cms were introduced into the tail and used as climb stoppers. An adhesive tape of length 17cm were cut for each mouse and at 2 cm from one end a mark was made. The 2cm marked portion of the tape was applied to the tip of the tail leaving 2-3 mm free and it was adhered securely. It was done for all the six animals in a group consecutively .Care was taken so that the tape is strong enough to hold the mouse's weight, and not too sticky while removing. The camera with a timer set up was positioned in a way so that the view of the tail suspension box was not obscured and the recording would not be interrupted. All the six mice in the group were suspended back to back by attaching the free end of the adhesive tape that measures 15 cm to the hook at the roof of all six compartments.

Without any interruption for the next six minutes the immobility time was recorded using the camera that was already positioned. The recordings were

saved at the end of six minutes. The animals were taken off the chamber; the tapes were removed with proper care. They were placed back into their cage and observed for a week.

TAIL SUSPENSION TEST –BEHAVIORAL ANALYSIS[56]

Assessment of the behaviour of all animals was done from the video recordings. Total observation period was six minutes. Immobility time was assessed. Important facet of this assessment was to differentiate between mobile and immobile state of the animal. Behaviours that were associated with escapism like all four limb movements, attempt to touch the side walls, shaking of the body, running like movements were considered as mobility. While small movements involving forelimb without any hind limb involvement, movements due to oscillatory movement of the tape because of momentum gained by previous motion of the animal were considered as immobility. We had used the whole period of six minutes for assessment, because in general in tail suspension test the mice tend to be immobile more during the early period of the test. The immobility period was recorded by this method in all the groups after the corresponding treatment, and the scores were analysed statistically.

RESULT

RESULTS

In this study the results of the tail suspension test of various groups are as follows:

Tail suspension test for control group

The control group received distilled water in addition to normal feed and water. Tail suspension test was conducted one hour after drug administration on day 7 and day 14 .The duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. It is expressed as Mean± S.D. The mean immobility period in seconds on day 7 and day 14 were 198.83 ± 14.95. and 194.33 ± 11.48

Table 1: TST result for control group

Day	**Control group immobility period in seconds**						**Mean ±S.D.**
	1	**2**	**3**	**4**	**5**	**6**	
Day 7	205	192	175	220	203	198	198.83 ± 14.95
Day 14	181	211	187	202	199	186	194.33 ± 11.48

Tail suspension test for standard group

The standard group received fluoxetin 20mg/kg in addition to normal feed and water. Tail suspension test was conducted one hour after drug therapy on day 7 and day 14 .The duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. It is expressed as Mean ± S.D. The mean immobility period in seconds on day 7 was 120.16 ± 19.06 and on day 14 it was 126.83 ± 13.4.

Table 2: TST for standard group

Day	standard group immobility period in seconds						Mean ±S.D.
	1	2	3	4	5	6	
Day 7	109	121	110	105	157	119	120.16±19.06
Day 14	144	115	117	134	138	113	126.83 ± 13.4

Tail suspension test for test group I

The mice in the test group- I received tablet Rosuvastatin 10 mg/kg in addition to normal feed and water. Tail suspension test was conducted one hour after drug therapy on day 7 and day 14, the duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. It is expressed as Mean ± S.D. The mean immobility period in seconds on day 7 was 131.83 ±25.76 and on day 14 it was 128.83 ± 12.57.

Table 3: TST result for test group I

Day	Test group- I immobility period in seconds						Mean ±S.D.
	1	2	3	4	5	6	
Day 7	142	115	96	155	120	163	131.83±25.76
Day 14	133	108	126	130	147	129	128.83 ± 12.57

Tail suspension test result for test group II

The mice in the test group-II received tablet Atrovastatin 10mg/kg in addition to normal feed and water. Tail suspension test was conducted one hour after drug therapy on day 7 and day 14. The duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. It is expressed as Mean± S.D. The mean immobility period in seconds on day 7 was 141.5±15.69 and on day 14 it was 145.5±11.11.

Table 4: TST result for test group II

Day	Test group- II immobility period in seconds						Mean ±S.D.
	1	2	3	4	5	6	
Day 7	144	118	162	132	154	139	141.5±15.69
Day 14	136	142	155	147	161	132	145.5±11.11

Tail suspension test result for test group III

The mice in the test group- III received Tablet. Simvastatin 10mg/kg, in addition to normal feed and water. Tail suspension test was conducted one hour after drug therapy with on day 7 and day 14 .The duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. It is expressed as Mean ± S.D. The mean immobility period in seconds on day 7 was 138.33±16.71 and on day 14 it was 132.5±15.1

Table 5: TST result for test group III

Day	Test group- III immobility period in seconds						Mean ±S.D.
	1	2	3	4	5	6	
Day 7	134	148	159	126	114	149	138.33±16.71
Day 14	122	`149	124	133	152	115	132.5±15.1

Results were analysed using the following statistical test.

IBM SPSS (statistical package for social sciences) software version 20 was used for statistical analysis.

Statistical test used in this experiment

One-way ANOVA (analysis of variance)

In this study five independent groups were compared, so ANOVA was used for analysis which is an extension of student t test (which is for the comparison of two groups). One-way ANOVA was used because there is a independent variable and single dependent variable. It was used to identify statistical difference between the mean immobility periods of the five independent groups. There by it disproves "null hypothesis".

POST HOC TEST

Bonferroni post hoc test:

Once it was resolved that that there was difference exists between the means among the five groups, to determine where the difference exist Bonferroni post hoc test was applied. It is most commonly used where an array of paired comparison are made i.e. one group is compared with all the other groups.

TABLE: 6 Mean ± SD immobility period in seconds on Day 7 and Day 14 in all groups

DAY	CONTROL	STANDARD	TEST I	TEST II	TEST III
Day 7	198.8±14.9	120.1±19**	131.8±25.7**	141.5±15.6**	138.33±16.7**
Day 14	194.3 ± 11.4	126.8 ±13.4**	128.8± 12.5**	145.5±11.11**	132.5±15.1**

**: Highly significant < 0.01

Result of One Way ANOVA and Post Hoc Test

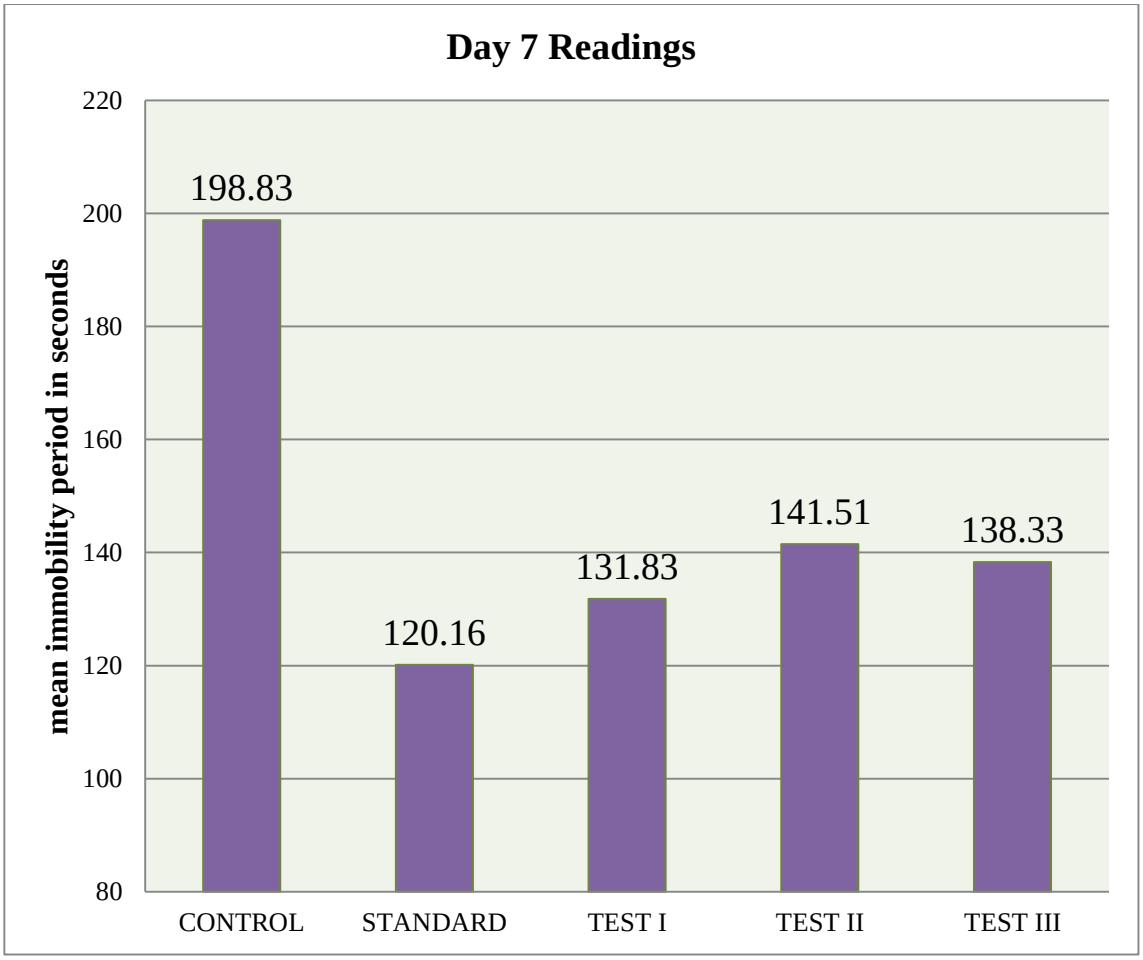
When the day 7 and day 14 results were compared and analyzed by one way anova. There is a difference in the mean immobility time between groups. F value is significant (<0.05) for the degree of freedom.

Post Hoc Test

After anova identified a significant difference, in order to find where the difference exist Bonferroni post hoc test were used.

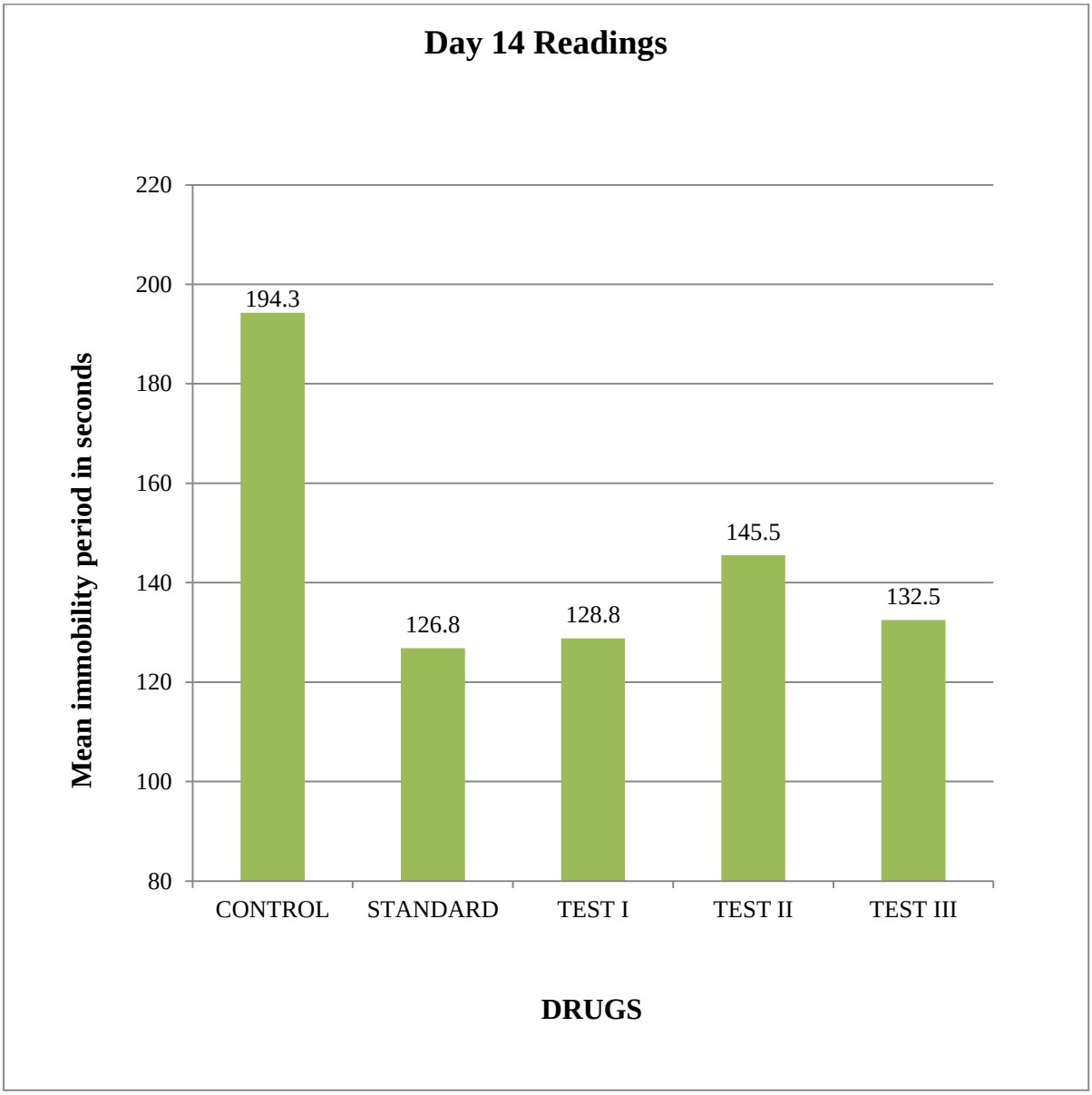
It showed there is a significant difference in the immobility period of standard and all three test groups on day 7 when compared with control group.

The mean immobility period on day 7 in all groups

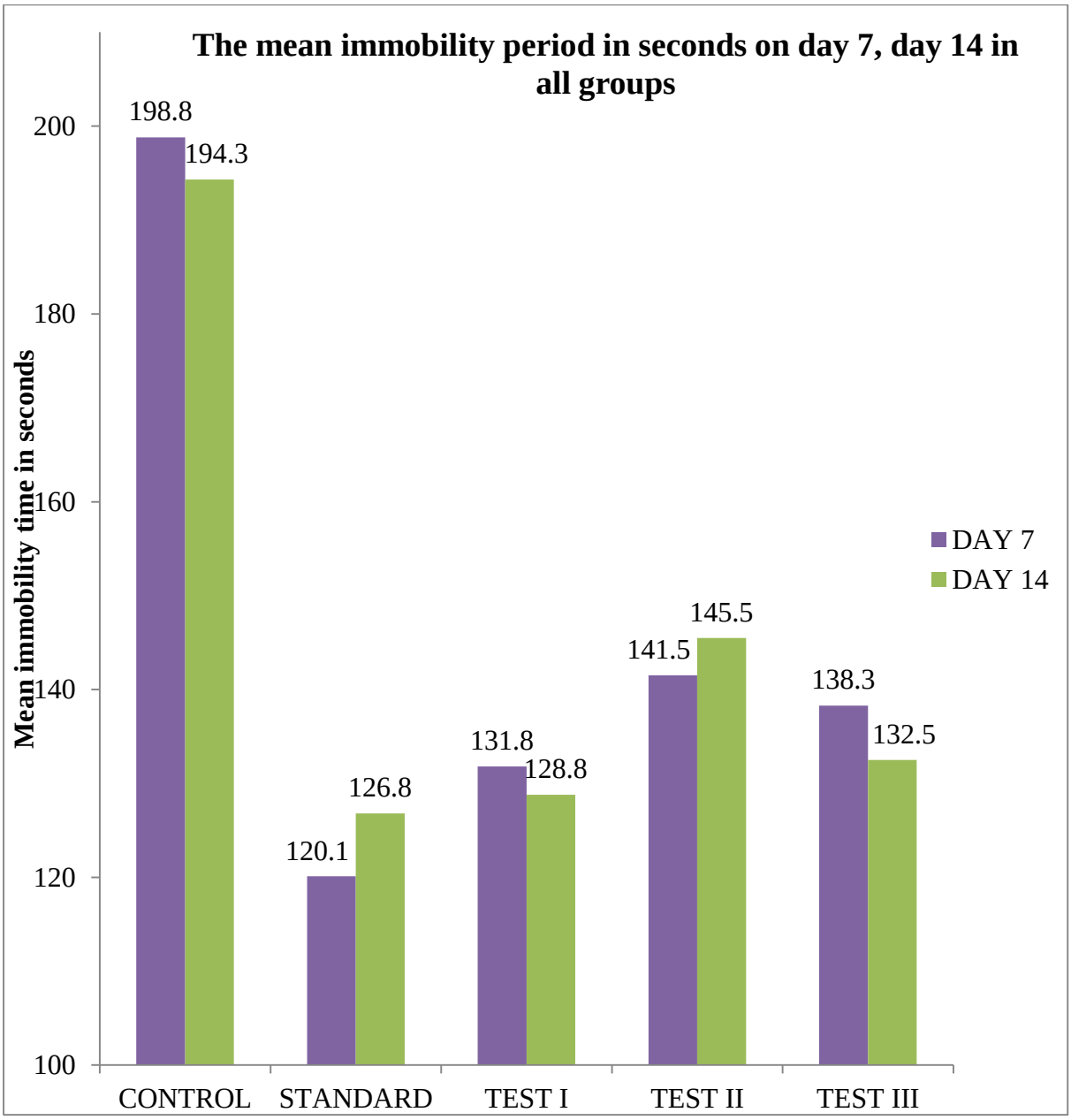


Similar to day 7, day 14 results of post hoc test exhibited a significant difference between control and all the other groups.

Figure: The mean immobility period on day 14 in all groups



Standard, test I, Test II, test III were highly significant ** when compared with control on day 14



Both on day 7 and day 14, there is a significant difference in the immobility period when we compare control with all the other groups. The difference is not significant when we compare the corresponding groups on day 7 and 14.

DISCUSSION

DISCUSSION

Depression is a highly prevalent psychiatric illness globally; based on the recent studies prevalence is expected to increase. It affects all age groups like teenagers, adults and old age of both sexes. The incidence is more among women. And it's on the rise among teenagers. It's the prime reason for DALY succeeding cardio vascular disease.

In patients affected by depression it augments the underlying medical illness; chronic medical illness is also associated with depression. The incidence of suicidal attempt is around 50%; 15% of those cohort die due to suicide[57]. Thereby the disease has to be corrected effectively and promptly.

With better understanding of the pathogenesis of depression, it is a treatable disease. There are certain pitfalls in the pharmacotherapy of depression like lack of efficacy, late onset of therapeutic effectiveness, adverse reaction, and non compliance. So the necessity for newer drugs or a newer target is the need of the hour

SSRIs are the commonly prescribed antidepressant. Statin is a hypolipidemic agent which is widely used. There is ample number of examples in the drug history where the drug in its post marketing surveillance was found to have new usages. Statin is an enigmatic drug because of its pleiotropic effect which makes it, a suitable candidate for investigational studies. In this study Statin was evaluated for its antidepressant effect in Swiss albino mice by tail suspension method.

Among the methods for evaluating antidepressants, tail suspension test is extensively used behaviour model. It has higher sensitivity and predictability. It is also a well established model for drugs acting through SERT.

In this study antidepressant property of statin was evaluated using tail suspension test by observing the changes in the immobility period. The variation in the immobility period was compared between groups. On day 7, in the tail suspension test there was a significant decrease in the duration of immobility in the standard group when compared with the control group. P value was < 0.01 level. The changes in the immobility period among the three test groups were also significant at 0.01 levels when compared with the control. There was no significant difference among the three test groups i.e. rosuvastatin, atrovastatin, simvastatin. There is also no statistical difference between the standard and the test groups.

On the day 14 also there was a significant decrease in immobility period in the standard when compared with the control. P value was < 0.0l. The mice were highly mobile in the test groups as well; here also the difference was statistically significant at 0.01 levels when compared with control. Similar to day 7 value there was no statistical significance when compared among the three test groups or the test group with standard. By taking into account the values of day 7 and 14, the study has established the antidepressant property of statin in animal model that was comparable with that of fluoxetine.

When we interpret the day7 and day 14 results there was no significant difference in the immobility period between the corresponding groups.

In a study completed by Perry F. Renshaw et al[58]. which is the prototype study for antidepressant effect of statin in animal models, they found that Lovastatin potentiate the sub effective dose of fluoxetine. In Sprague Dawley rats they used 5mg/kg of fluoxetine and 2mg/kg/day of lovastatin. Forced swim test was performed. Lovasatin along with flluoxetine decreased the percentage of immobility period. Lovastatin alone had no antidepressant effect. In our test statin alone exhibited antidepressant property which was comparable with that of fluoxetine. The variation may be due to recommended dose of the statin used in the study when compared to the potentiating dose of lovastatin. In another study by Pao-Yen Lin et al[59]., it was found that simvastatin exhibit antidepressant property in rats which were exposed to chronic stress. Dose of 5 and 10 mg/ kg were used for 14 days. The antidepressant effect was comparable to that of imipramine, but there was no difference in the response between the two doses. In this study, in addition to simvastatin other statins like atrovastatin and rosuvastatin also showed antidepressant effect on par with fluoxetine.

Although animal studies support a beneficial role of statins in attenuating depressive symptoms, the mechanism by which it exhibits the antidepressant effects is speculative. In a study done by Stefan John et al[60]. statins increased the level of nitric oxide when tried in patient with hypercholesterolemia. In this study, fluvastatin treated group exhibited a significant enhancement in the

acetylcholine-induced increase in blood flow in the forearm when compared with the baseline. After infusion of NG-monomethyl-L-arginine (L-NMMA) the NO synthase inhibitor, there was a graded decrease in blood flow with increasing doses. Thereby statin increases NO synthesis. In a study by Aiwu Cheng et al[61]. it was demonstrated that BDNF increases nNOS production during differentiation of neural progenitor cells and nitric oxide mediates the BDNF activity. So nitric oxide is involved in a positive feedback chain with BDNF. In a study by Xiaoguang Han et al[62]., simvastatin treatment after trauma reduced the myelin loss, increased the expression of trophic factors like BDNF and GDNF, and fastens functional recovery. Thereby we can correlate that depression attenuating activity may be due to NO and BDNF cycle. In a study done by Mitani et al. [63] increased levels of glutamate in blood were found in patients with depressive disorder; glutamate is a excitatory neurotransmitter and its considered to be the mediator of exitotoxicity in degenerative disorder. This activity of glutamate is mediated through NMDA receptor. In a study done by Jovita Ponce et al statins decrease the NMDA receptor mediated activity, then they play a role in decreasing the excitotoxicity by glutamate[64].

So statins promote the neurotropic mediated activity and counter the glutamate mediated excitotoxicity. In addition to this, statins also exhibit anti inflammatory, immunomodulatory and anti oxidant property. It decreases the transcription of various inflammatory mediators and modulates the adhesion process at various points by targeting both leukocytes and endothelium. They inhibit cell chemokine expression, integrin expression, integrin activation in

leukocytes, stimulated endothelial cells, and monocytes[65]. The pro-inflammatory mediators IFN-α, IL-1β, NF-κB, IL-6 decreases the nerve growth factor; similarly a decrease in anti-inflammatory mediators like IL-10 also associated with decrease in nerve growth factor. Thereby inflammation as a causative factor in depression provides newer targets against the disease. Anti –Inflammatory and immunomodulatory property of statin go hand in hand. Depressive disorder is associated with immunological abnormalities like lymphocyte mediated increase of CRF, Cytokines and Interleukins[66]. The oxidative stress play an important role in depression .In a study by Kyoko meji et al[67]. they ensures Pitavastatin enhances the Cu/Zn Super Oxide Dismutase (SOD) and PPAR-gamma expression, they also inhibit NAD(P)H oxidase activity .This explains antioxidant property of statin. There is a paradigm shift in the pathogenesis of depression from monoamine hypothesis to neurotropic hypothesis. Statin seems to counter the various pathological factors of depression through it's pleiotropic effect.

Finally the vascular depression which happens in old age; it is characterised by hyper intense lesion on MRI (magnetic resonance imaging) and high degree of resistance to antidepressants. The pathophysiological basis is inflammation and hemodynamic changes. It results in altered function due to hypoperfusion which will end up in depression[68]. Statin due to its pleiotropic effect may reduce the variation in microvascular resistance. In addition to this, inhibition of Rac1 phrenylation in endothelial cells induces the expression of a wide range of genes implicated in neurovascular protection[69].

Overall, the precise mechanism by which statin exhibit antidepressant property is not clear. Various neurotropic activity and anti-inflammatory, immunomodulatory, antioxidant property acts in a cumulative manner to improve functional outcome and may contribute to the overall antidepressant activity of statins.

CONCLUSION

CONCLUSION

Major depressive illness is a heterogeneous disorder with high morbidity and mortality. The heterogeneity is because of its integrated etiological model. The disease is often misdiagnosed and undertreated. With the availability of newer antidepressants the disease is very much treatable. There are certain snags in the pharmacotherapy of depression like therapeutic lag, decreased compliance, adverse reaction and also 30% of patients remain non responders to the available antidepressants.

In this study statin was evaluated for its antidepressant property by tail suspension method. It exhibited antidepressant effect which was statistically significant and was comparable with that of fluoxetine. Thereby the study objective was completed.

Depression is frequently associated with medical conditions like cardiovascular illness arthritis, stroke. On the other side depression is also an individual risk factor for cardio and cerebrovascular diseases, the use of statin can be more justified in these conditions. Statins has the potential to be used as an adjuvant in the treatment of depression, particularly in the treatment of resistant depression and vascular depression.

Further animal studies involving large number of animals, other models of depression are needed. It can also help us to know the target through which statins act and based on targets new lead molecules can be generated.

ANNEXURES

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MASTER CHART

MASTER CHART

Day 7 immobility time in seconds

S.NO	Control	standard	Test I	Test II	Test III
Mouse-1	205	109	142	144	134
Mouse-2	192	121	115	118	148
Mouse-3	175	110	96	162	159
Mouse-4	220	105	155	132	126
Mouse-5	203	157	120	154	114
Mouse-6	198	119	163	139	149

Day 14 immobility time in seconds

S.NO	Control	standard	Test I	Test II	Test III
Mouse-1	181	144	133	136	122
Mouse-2	211	115	108	142	149
Mouse-3	187	117	126	155	124
Mouse-4	202	134	130	147	133
Mouse-5	199	138	147	161	152
Mouse-6	186	113	129	132	115

ABBREVIATION

WHO	World Health Organisation
CBT	Cognitive Behaviour Therapy
TCA	Tricyclic Antidepressants
MAOIs	Monoamine Oxidase Inhibitors
SSRIs	Selective Serotonin Reuptake Inhibitors
SNRIs	Serotonin nor adrenaline Reuptake Inhibitors
MMD	Major Mood Disorder
DALY	Disability adjusted life year
PPD	Post Partum Depression
5-HT	5-Hydroxy Tryptamine
NA	Nor adrenaline
CSF	Cerebro Spinal Fluid
CRH	Corticotrophin-releasing hormone
TRH	TSH releasing hormone
ACTH	Adrenocorticotrophic hormone
BDNF	Brain-Derived Neurotrophic Factor
AIDS	Acquired Immuno Deficiency Syndrome
DSM-V	Diagnostic and Statistical Manual of Mental Disorders-V
PMDD	Premenstrual Dysphoric Disorder
ECT	Electro Convulsive Therapy
RIMA	Reversible MAO-A-selective inhibitors
IPT	Inter Personal Therapy

FDA	Federal Drug Agency
TMS	Transcranial Magnetic Stimulation
SERT	Serotonin reuptake transporter
CYP	Cytochrome p 450
OCD	Obsessive Compulsive Disorder.
CTZ	Chemoreceptor Trigger Zone.
NET	Nor epinephrine transporter
NMDA	N-Methyl-D-aspartate
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
MIF-1	Melanocyte inhibiting factor- 1
LDL	Low density lipoprotein
HMG CO-A	3-Hydroxy- 3 Methyl Glutaryl Coenzyme A.
NO	Nitric oxide
EPC	Endothelial Progenitor Cell
eNOS	Endothelial nitric oxide synthase
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
INF	INTERFERON
MCH II	Major Histocompatibility Complex II
MMP	Matrix metalloproteinase
NADPH	Nicotinamide adenine dinucleotide phosphate
BMP	Bone morphogenic protein
CPK	Creatine phosphokinase
CVD	Cardio Vascular Diseases

MI	Myocardial Infarction
CPCSEA	Committee for the Purpose of Controlled Supervision of Experimentation on Animals
ANOVA	Analysis of Variance
L-NMMA	NG-monomethyl-L-arginine
GDNF	Glial Derived NeurotropicFactor.
IL	Interleukin
SOD	Super Oxide Dismutase
PPAR	Peroxisome Proliferator-Activated Receptor.
MRI	Magnetic Resonance Imaging.

ETHICAL
CLEARANCE LETTER

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ANTI PLAGIARISM CERTIFICATE

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